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[54] **FUMONISIN DETOXIFICATION COMPOSITIONS AND METHODS**

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Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 289,595, Aug. 12, 1994, abandoned.

[51] **Int. Cl.**^{6} **A01H 5/00**; C12N 9/16

[52] **U.S. Cl.** **800/205**; 435/172.3; 435/172.1; 435/183; 435/196; 47/58

[58] **Field of Search** ..... 800/208, 200; 47/58, DIG. 1; 435/172.3, 172.1, 196, 182, 195, 197; 424/93.1; 536/27.1, 24.1

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[57] **ABSTRACT**

Methods for identifying organisms capable of degrading fumonisin. Fumonisin can be incorporated into culture medium for selection of organisms resistant to fumonisin and/or capable of growing on flumonisin as a sole carbon source. Using this method, several organisms have been identified. These organisms can be used to isolate the enzyme and the gene responsible for conferring fumonisin-resistance. The gene can be cloned and inserted into a suitable expression vector so that the protein can be further characterized. Additionally, the DNA encoding for furaonisin-resistance can be used to transform plant cells normally susceptible to Fusarium or other toxin-producing fungus infection. Plants can be regenerated from the transformed plant cells. In this way, a transgenic plant can be produced with the capability of degrading fumonisin, as well as with the capability of producing the degrading enzymes. Methods for detoxification in grain processing and in animal feed and rumen microbes are also disclosed.

4 Claims, 2 Drawing Sheets

FIGURE 1

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ACTAGTGGAT CATTGCATTG GCTGGCGGAC TGGCGCGCCG ATAGTCGTTG     1
CGATGGTCGC GAGAATAAGC GTGCGAAGTG GGAGGATGTG AAGATGGGGG    51
CCAGGAGTAT GTGTGCGGGA CGGTTCGGAC GCTTCTGCAT TGGCTTGGCT   101
TCATCGGTTG CCGTGACTCT AGGGGGAGCC TCCGCCGCCG GCGCGGCAAC   151
CGCGACGGAT TTTCCGGTCC GCAGGACCGA TCTGGGCCAG GTTCAGGGAC   201
TGGCCGGGGA CGTGATGAGC TTTCGCGGAA TACCCTATGC AGCGCCGCCG   251
GTGGGCGGGC TGCGTTGGAA GCCGCCCCAA CACGCCCGGC CCTGGGCGGG   301
CGTTCGCCCC GCCACCCAAT TTGGCTCCGA CTGCTTCGGC GCGGCCTATC   351
TTCGCAAAGG CAGCCTCGCC CCCGGCGTGA GCGAGGACTG TCTTTACCTC   401
AACGTATGGG CGCCGTCAGG CGCTAAACCC GGCCAGTACC CCGTCATGGT   451
CTGGGTCTAC GGCGGCGGCT TCGCCGGCGG CACGGCCGCC ATGCCCTACT   501
ACGACGGCGA GGCGCTTGCG CGACAGGGCG TCGTCGTGGT GACGTTTAAC   551
TATCGGACGA ACATCCTGGG CTTTTTCGCC CATCCTGGTC TCTCGCGCGA   601
GAGCCCCACC GGAACTTCGG GCAACTACGG CCTACTCGAC ATTCTCGCCG   651
CTCTTCGGTG GGTGCAGAGC AACGCCCGCG CCTTCGGAGG GGACCCCGGC   701
CGAGTGACGG TCTTTGGTGA ATCGGCCGGA GCGAGCGCGA TCGGACTTCT   751
GCTCACCTCG CCGCTGAGCA AGGGTCTCTT CCGTGGCGCT ATCCTCGAAA   801
GTCCAGGGCT GACGCGACCG CTCGCGACGC TCGCCGACAG CGCCGCCTCG   851
GGCGAGCGCC TCGACGCCGA TCTTTCGCGA CTGCGCTCGA CCGACCCAGC   901
CACCCTGATG GCGCGCGCCG ACGCGGCCCG CCCGGCATCG CGGGACCTGC   951
GCAGGCCGCG TCCGACCGGA CCGATCGTCG ATGGCCATGT GCTGCCGCAG  1001
ACCGACAGCG CGGCGATCGC GGCGGGGCAG CTGGCGCCGG TTCGGGTCCT  1051
GATCGGAACC AATGCCGACG AAGGCCGCGC CTTCCTCGGG CGCGCGCCGA  1101
TGGAGACGCC AGCGGACTAC CAAGCCTATC TGGAGGCGCA GTTTGGCGAC  1151
CAAGCCGCCG CCGTGGCGGC GTGCTATCCC CTCGACGGCC GGGCCACGCC  1201
CAAGGAAATG GTCGCGCGCA TCTTCGGCGA CAATCAGTTC AATCGGGGGG  1251
TCTCGGCCTT CTCGGAAGCG CTTGTGCGCC AGGGCGCGCC CGTGTGGCGT  1301
TATCAGTTCA ACGGTAATAC CGAGGGTGGA AGAGCGCCGG CTACCCACGG  1351
AGCCGAAATT CCCTACGTTT TCGGGGTGTT CAAGCTCGAC GAGTTGGGTC  1401
TGTTCGATTG GCCGCCCGAG GGGCCCACGC CCGCCGACCG TGCGCTGGGC  1451
CAACTGATGT CCTCCGCCTG GGTCCGGTTC GCCAAGAATG GCGACCCCGC  1501
CGGGGACGCC CTTACCTGGC CTGCCTATTC TACGGGCAAG TCGACCATGA  1551
CATTCGGTCC CGAGGGCCGC GCGGCGGTGG TGTCGCCCGG ACCTTCCATC  1601
CCCCCTTGCG CGGATGGCGC CAAGGCGGGG TGACGCCGTC GACGATGGCG  1651
TGACGACGGT CGAGGCGATG TTCTCGATCT GGAGTCCGCG CCGCCTCGAT  1701
TTGCGTCGTC TCCGGCGCTC AGACGAACGC CCCAGTTCCA TCCACACAGT  1751
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FIGURE 2

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MGARSMCAGR FGRECIGLAS SVAVTLGGAS AAGAATATDF PVRRTDLGQV      1
QGLAGDVMSF RGIPYAAPPV GGLRWKPPQH ARPWAGVRPA TQFGSDCFGA     51
AYLRKGSLAP GVSEDCLYLN VWAPSGAKPG QYPVMVWVYG GGFAGGTAAM    101
PYYDGEALAR QGVVVVTFNY RTNILGFFAH PGLSRESPTG TSGNYGLLDI    151
LAALRWVQSN ARAFGGDPGR VTVFGESAGA SAIGLLLTSP LSKGLFRGAI    201
LESPGLTRPL ATLADSAASG ERLDADLSRL RSTDPATLMA RADAARPASR    251
DLRRPRPTGP IVDGHVLPQT DSAAIAAGQL APVRVLIGTN ADEGRAFLGR    301
APMETPADYQ AYLEAQFGDQ AAAVAACYPL DGRATPKEMV ARIFGDNQFN    351
RGVSAFSEAL VRQGAPVWRY QFNGNTEGGR APATHGAEIP YVFGVFKLDE    401
LGLFDWPPEG PTPADRALGQ LMSSAWVRFA KNGDPAGDAL TWPAYSTGKS    451
TMTFGPEGRA AVVSPGPSIP PCADGAKAG*                          501
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FUMONISIN DETOXIFICATION COMPOSITIONS AND METHODS

This is a continuation-in-part of prior U.S. application Ser. No. 08/289,595, filed Aug. 12, 1994 now abandoned.

TECHNICAL FIELD

The present invention relates generally to the detection and isolation of fumonisin resistant organisms and to compositions and methods for the in vivo detoxification or degradation of fumonisin. This method has broad application in agricultural biotechnology and crop agriculture and in the improvement of food grain quality.

BACKGROUND OF THE INVENTION

Fungal diseases are common problems in crop agriculture. Many strides have been made against plant diseases as exemplified by the use of hybrid plants, pesticides and improved agricultural practices. However, as any grower or home gardener can attest, the problems of fungal plant disease continue to cause difficulties in plant cultivation. Thus, there is a continuing need for new methods and materials for solving the problems caused by fungal diseases of plants. These problems can be met through a variety of approaches. For example, the infectious organisms can be controlled through the use of agents that are selectively biocidal for the pathogens. Another method is interference with the mechanism by which the pathogen invades the host crop plant. Yet another method, in the case of pathogens that cause crop losses, is interference with the mechanism by which the pathogen causes injury to the host crop plant. Still another method, in the case of pathogens that produce toxins that are undesirable to mammals or other animals that feed on the crop plants, is interference with toxin production, storage, or activity. This invention falls into the latter two categories.

Since their discovery and structural elucidation in 1988. (Bezuidenhout S., Gelderblom W., Gorst-Allman C., Horak R., Marasas W., Spiteller B., Vleggaar R. (1988) "Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*." Journal Chem Soc, Chem Commun 1988: 743–745), fumonisins have been recognized as a potentially serious problem in maize-fed livestock. They are linked to several animal toxicoses including leukoencephalomalacia, (Marasas W. F. O., Kellerman T. S., Gelderblom W. C. A., Coetzer J. A. W., Thiel P. (1988) "Leukoencephalomalacia in a horse induced by fimonisin B-1 isolated from *Fusarium moniliforme*." Onderstepoort Journal of Veterinary Research 55: 197–204; Wilson T. M., Ledet A. E., Owens D. L., Rice L. G., Nelson H. A. (1990) "Experimental liver disease in ponies associated with the ingestion of a corn-based ration naturally contaminated with fumonisin B_1, " American Association of Veterinary Laboratory Diagnosticians: Abstracts 33rd Annual Meeting, Denver, Colorado, Oct. 7–9, 1990., Madison, Wis., U.S.A.) and porcine pulmonary edema (Colvin B. M., Harrison L. R. (1992) "Fumonisin-Induced Pulmonary Edema and Hydrothorax in Swine." Mycopathologia 117: 79–82) and are also suspected carcinogens (Geary W. (1971) Coord Chem Rev 7: 81; Gelderblom W. C. A., Kriek N. P. J., Marasas W. F. O., Thiel P. G. (1991) "Toxicity and Carcinogenicity of the Fusarium-Moniliforme Metabolite, Fumonisin-B1, in Rats." Carcinogenesis 12: 1247–1251; Gelderblom W. C. A., Semple E., Marasas W. F. O., Farber E. (1992) "The Cancer-Initiating Potential of the Fumonisin-B Mycotoxins." Carcinogenesis 13: 433–437). Fusarium isolates in section Liseola produce

fumonisins in culture at levels from 2 to 22 4000 ppm .(Leslie J., Plattner R., Desjardins A., Klittich C. (1992) "Fumonisin B1 production by strains from different mating populations of *Gibberella fujikoroi*(Fusarium section Liseola). " Phytopathology 82: 341–345). Isolates from maize (predominantly mating population A) are among the highest producers (Leslie et al., supra). Fumonisin levels detected in field-grown maize have fluctuated widely depending on location and growing season, but both preharvest and postharvest surveys of field maize have indicated that the potential for high levels of fumonisins exists. (Murphy P. A., Rice L. G., Ross P. F. (1993) "Fumonisin-B1, Fumonisin-B2, and Fumonisin-B3 content of Iowa, Wisconsin, and Illinois corn and corn screenings." J Agr Food Chem 41: 263–266). Surveys of food and feed products have also detected fumonisin. (Holcomb M., Thompson H. C. Jr., Hankins L. J. (1993) "Analysis of fumonisin B-1 in rodent feed by gradient elution HPLC using precolumn derivation with FMOC and fluorescence detection." J Agr Food Chem 41: 764–767; Hopmans E. C., Murphy P. A. (1993) "Detection of Fumonisin-B(1), Fumonisin-B(2), and Fumonisin-B(3) and hydrolyzed Fumonisin-B(1) in Corn-Containing foods." J Agr Food Chem 41: 1655–1658; Sydenham E. W., Shephard G. S., Thiel P. G., Marasas W. F. O., Stockenstrom S. (1991) "Fumonisin Contamination of Commercial Corn-Based Human Foodstuffs." J Agr Food Chem 39: 2014–2018). The etiology of Fusarium ear mold is poorly understood, although physical damage to the ear and certain environmental conditions can contribute to its occurrence(Nelson P. E. (1992) "Taxonomy and Biology of *Fusarium moniliforme*." Mycopathologia 117: 29–36). Fusarium can be isolated from most field grown maize, even when no visible mold is present. The relationship between seedling infection and stalk and ear diseases caused by Fusarium is not clear. Genetic resistance to visible kernel mold has been identified.(Gendloff E., Rossman E., Casale W., Isleib T., Hart P. (1986) "Components of resistance to Fusarium ear rot in field corn." Phytopathology 76: 684–688; Holley R. N., Hamilton P. B., Goodman M. M. (1989) "Evaluation of tropical maize germplasm for resistance to kernel colonization by *Fusarium moniliforme*." Plant Dis 73: 578–580), but the relationship to visible mold to fumonisin production has yet to be elucidated.

Fumonisins have been shown in in vitro mammalian cell studies to inhibit sphingolipid biosynthesis through inhibition of the enzyme sphinganine acyl transferase (Norred W. P., Wang E., Yoo H., Riley R. T., Merrill A. H. (1992) "In vitro toxicology of fumonisins and the mechanistic implications." Mycopatliologia 117: 73–78; Wang E., Norred W. Bacon C., Riley R., Merrill A. Jr. (1991) "Inhibition of sphingolipid biosynthesis by fumonisins: implications for diseases associated with *Fusarium moniliforme*." J Biol Chem 266: 14486; Yoo H. S., Norred W. P., Wang E., Merrill A. H., Riley R. T. (1992) "Fumonisin Inhibition of de Novo Sphingolipid Biosynthesis and Cytotoxicity Are Correlated in LLC-PK1 Cells." Toxicol Appl Pharmacol 114: 9–15), resulting in the accumulation of the precursor sphinganine. It is likely that inhibition of this pathway accounts for at least some of fumonisin's toxicity, and support for this comes from measures of sphinganine:sphingosine ratios in animals fed purified fumonisin.(Wang E., Ross P. F., Wilson T. M., Riley R. T., Merrill A. H. (1992) "Increases in Serum Sphingosine and Sphinganine and Decreases in Complex Sphingolipids in Ponies Given Feed Containing Fumonisins, Mycotoxins Produced by *Fusarium moniliforme*." J Nutr 122: 1706–1716). Fumonisins also affect plant cell growth (Abbas H. K., Boyette C. D. (1992) "Phytotoxicity of

fumonisin B_1, on weed and crop species." Weed Technol 6: 548–552; Vanasch M. A. J., Rijkenberg F. H. J., Coutinho T. A. (1992) "Phytotoxicity of fumonisin B $_1$, moniliformin, and t-2 toxin to corn callus cultures." Phytopathology 82: 1330–1332; Vesonder R. F., Peterson R. E., Labeda D, Abbas H. K. (1992) "Comparative phytotoxicity of the fumonisins, AAL-Toxin and yeast sphingolipids in *Lemna minor L.* (Duckweed)." Arch Environ Contam Toxicol 23: 464–467). Kuti et al. "Effect of fumonisin B1 on virulence of Fusarium species isolated from tomato plants." (Abstract, Annual Meeting American Phytopathological Society, Memphis, Tenn.: ASP Press 1993) reported on the ability of exogenously added fumonisins to accelerate disease development and increase sporulation of *Fusarium moniliforme* and *F. oxysporum* on tomato.

The toxicity of fumonisins and their potential widespread occurrence in food and feed makes it imperative to find detoxification or elimination strategies to remove the compound from the food chain.

DISCLOSURE OF THE INVENTION

The present invention is based on the discovery of organisms with the ability to degrade the mycotoxin fumonisin. In a search for a biological means of detoxifying fumonisins, we have isolated from field-grown maize kernels several dematiaceous hyphomycetes capable of growing on fumonisin B_1 or B_2 (FB_1 or FB_2) as a sole carbon source, degrading it partially or completely in the process. One species, identified as *Exophiala spinifera*, a "black yeast", was recovered from maize seed from diverse locations in the southeastern and south central U.S. A related species, *Rhinocladiella atrovirens*; was isolated from seed originating in both Iowa and Georgia. We also isolated a bacterium, believed to be a Xanthomonas or Sphingomonas species, designated isolate 2412.1, from a field-grown maize stalk sample from Johiston, Iowa. This bacterium also showed growth on FB_1 as a sole carbon source, and since its taxonomy is not certain we have deposited the strain with the American Type Culture Collection (ATCC) and it is referred to herein by its ATCC deposit number, 55552. We have also deposited enzyme-active strains of *Exophiala spinifera* (ATCC 74269) and *Rhinocladiella atrovirens*(ATCC 74270).

All isolates showed the capability to degrade FB_1 in liquid culture. By "degrade" is simply meant that the enzyme is capable of using fumonisin as a substrate and converting it to a different chemical structure. However, our studies indicate that the resulting compounds are less toxic than the fumonisins themselves.

Overall, only 16 of 70 independent seed samples tested yielded degraders. However, several *E. spinifera isolates*, collected outside the U.S. from non-maize sources, were also found to metabolize fumonisins. Representative isolates of other Exophiala species tested (E. jeanselmi, E. salmonis, E. piscifera) did not degrade fumonisins, nor did non-maize Rhinocladiella isolates, including *R. atrovirens* and *R. anceps*, nor fungi associated with ear molds including *Fusarium moniliforine, F. graminearum, Aspergillus flavus* and *Diplodia maydis*. Fumonisin-metabolizing black yeasts were found to possess an inducible hydrolase activity that cleaves the tricarballylate esters of FB_1 , as monitored by C_{18}-thin layer chromatography (TLC) and fluorescence detection of amines. The identity of the resulting amino alcohol compound, designated AP_1, was verified by FAB-mass spectroscopy. The latter compound has utility as a chemical indicator of fumonisin metabolism. *E. spinifera* cultures further metabolized AP_1 to compounds of unknown identity that were not detectable by amine reagents on TLC. In sealed culture chambers, *E. spinfera* grown on ^{14}C FB, as a sole carbon source, released $^{14}CO_2$ as detected in 1N KOH-saturated filler paper strips, totaling percent of added label in 48 hours. Heat-killed cultures similarly incubated did not release appreciable $^{14}CO_2$. Thus, at least a portion of the fumonisin is fully metabolized by this fungus. Crude, cell-free culture filtrates of the *E. spinifera* isolate designated 2141.10 contained a heat-labile, protease-sensitive hydrolase activity attributed to an enzyme tentatively characterized as an esterase with specificity for tricarballylate esters of fumonisins and similar molecules such as AAL-toxin from *Alternaria alternata lycopersici*. This purified esterase is believed to be a new chemical entity, since no commercially available esterases tested were able to hydrolyze the tricarballylate esters of FB_1, suggesting a novel enzyme specificity produced by these fungi. Cell-free extracts of *E. spinfera* isolate 2141.10 also contain an AP1 catabolase capable of converting AP1 to a compound lacking a free amine group,possibly an aldehyde. These enzymes and genes coding for these enzymes, being involved in fumonisin degradation, have utility in detoxification of maize seed pre- or post-harvest.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is the nucleotide sequence including the open reading frame coding for the bacterial esterase gene (bases 94 to 1683) (SEQ ID NO:1).

FIG. 2 is a hypothetical amino acid sequence of the polypeptide encoded by bases 94 to 1683 of the nucleotide sequence (SEQ ID NO:2) of FIG. 1. Residues 1–38 represent the putative signal sequence. The polypeptide including the signal sequence has a calculated molecular weight of 55,026.68(529 residues), with a calculated pI of 8.70. The polypeptide without the putative signal sequence has a calculated molecular weight of 51,495.63(491 residues), with a calculated pI of 8.19.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides newly discovered enzymes capable of degrading and detoxifying fumonisins, produced by fermentation of one or more of *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium of ATCC 55552. The invention further comprises methods for making enzymes that are capable of detoxifying fumonisins, comprising the step of growing one or more of *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium ATCC 55552 in the presence of a fumonisin or the metabolite produced by action of the enzyme on a fumonisin. This invention further provides methods of detoxifying fumonisins, comprising the step of reacting them with an enzyme derived from *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium of ATCC 55552.

We have isolated and sequenced the gene that codes for the fumonisin-degrading enzyme from one of these organisms and provide the amino acid sequence of the enzyme here. It is known that genes encoding desired proteins can be identified, isolated, cloned and expressed in transgenic organisms, including several important crop plants. One commonly used method of gene transfer in plants involves the use of a disarmed form of the Ti plasmid of the soil bacterium *Agrobacterium tumefaciens*. *A. tumefaciens* is a plant pathogen that causes crown-gall tumors in infected

plants. Large plasmids, termed Ti- or tumor-inducing plasmids, are responsible for the oncogenicity of the bacterium as well as for the transfer of foreign DNA to the plant. Similarly, A. rhizogenes contains Ri- or root-inducing plasmids that induce root growth. Both plasmid types include a vir or virulence region that must be functional in order to transform wild-type cells to tumor cells.

Transformation results in the integration of another plasmid portion, termed the T- or transfer-DNA, into the nuclear genome of the transformed cells. Ri and Ti plasmids can be manipulated to allow insertion of foreign DNA, encoding a desired protein, into the T-DNA region. The foreign DNA can be transferred either via a vector bearing both the vir gene and the foreign gene or by a binary vector system consisting of two plasmids, one containing the vir gene and the other carrying the foreign gene. See, e.g., U.S. Pat. No. 4,658,082. Transformed plant cells can then be regenerated to produce varieties bearing the inserted gene. The production of transgenic, fumonisin-resistant plants will provide a useful and novel approach for the control of Fusarium-induced plant diseases.

This invention also provides a mechanism for selection of transformants: growth of plant cells in the presence of a Fusarium or its mycotoxin favors the survival of plant cells that have been transformed to express the coding sequence that codes for the enzyme of this invention and degrade the toxin. Thus, the coding sequence that codes for the enzyme of this invention can itself be used as a selectable marker, or as a scorable marker by measuring formation of the amino alcohol metabolite.

Another embodiment of the present invention is directed to a DNA construct comprising an expression cassette comprised of:

a) a DNA coding sequence for a polypeptide capable of degrading fumonisin; and

b) control sequences that are operably linked to the coding sequence whereby the coding sequence can be transcribed and translated in a host cell, and at least one of the DNA coding sequences or control sequences is heterologous to the host cell.

Preferred embodiments of the subject invention include a host cell stably transformed by a DNA construct as described above; and a method of producing a polypeptide of a recombinant gene comprising:

a) providing a population of these host cells; and

b) growing the population of cells under conditions whereby the polypeptide encoded by the coding sequence of the expression cassette is expressed.

In yet another embodiment, the present invention is directed to a transgenic plant capable of degrading fumonisin. In another embodiment., the transgenic plant is a maize plant capable of degrading fumonisin.

Another embodiment of the subject invention comprises a method of conferring fumonisin-resistance to a plant substantially without such resistance comprising transferring to the plant an expressible gene encoding a polypeptide capable of degrading fumonisin.

Thus, DNA encoding a protein able to inactivate fumonisin can be isolated and cloned in an appropriate vector and inserted into an organism normally sensitive to the Fusarium or its toxin. Organisms expressing the gene can be easily identified by their ability to degrade fumonisin. The protein capable of degrading fumonisin can be isolated and characterized using techniques well known in the art. Furthermore, the gene imparting fumonisin-resistance can be transferred into a suitable plasmid, such as into the T-DNA region of the Ti or Ri plasmid of the soil bacteria *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*, respectively. Plant tissue can be inoculated with the transformed bacteria. Additionally, plant tissues which have been co-cultivated with Agrobacterium spp can be incubated in the presence of fumonisin to select for fumonisin-degrading transgenic plants, i.e., the gene for fumonisin degradation can serve as a selectable marker. Thus, the inoculated tissue is regenerated to produce fumonisin-degrading transgenic plants.

Additionally, the present invention relates to ruminal microorganisms that have been genetically engineered with the genes imparting fumonisin resistance. These engineered ruminal microorganisms can then be added to feed for consumption by animals susceptible to fumonisin and structurally related mycotoxins.

Industrial Applicability

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., J. H. Langenheim and K. V. Thimann, Botany: Plant Biology and Its Relation to Human Affairs (1982) John Wiley; Cell Culture and Somatic Cell Genetics of Plants, Vol. 1 (I. K. Vasil, ed. 1984); R. V. Stanier, J. L. Ingraham, M. L. Wheelis, and P. R. Painter, The Microbial World, (1986) 5th Ed., Prentice-Hall; O. D. Dhringra and J. B. Sinclair, Basic Plant Pathology Methods, (1985) CRC Press; Maniatis, Fritsch & Sambrook, Molecular Cloning: A Laboratory Manual (1982); DNA Cloning, Vols. I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); and the series Methods in Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.).

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "microbe" is meant any microorganism (including both eucaryotic and procaryotic microorganisms), such as fungi, yeasts, bacteria, actinomycetes, algae and protozoa, as well as other unicellular structures capable of growth in culture.

A "fumonisin-producing microbe" is any microbe capable of producing the mycotoxin fumonisin or analogs thereof Such microbes are generally members of the fungal genus Fusarium, as well as recombinantly derived organisms which have been genetically altered to enable them to produce fumonisin or analogues thereof By "degrading fumonisin" is meant any modification to the fumonisin molecule which causes a decrease or loss in its toxic activity. Such a change can comprise cleavage of any of the various bonds, oxidation, reduction, the addition or deletion of a chemical moiety, or any other change that affects the activity of the molecule. In a preferred embodiment, the modification includes hydrolysis of the ester linkage in the molecule as a first step. Furthermore, chemically altered fumonisin can be isolated from cultures of microbes that produce an enzyme of this invention, such as by growing the organisms on media containing radioactively-labeled fumonisin, tracing the label, and isolating the degraded toxin for further study. The degraded fumonisin can be compared to the active compound for its phytotoxicity or mammalian toxicity in known sensitive species, such as porcines and equines. Such toxicity assays are known in the art. For example, in

plants a whole leaf bioassay can be used in which solutions of the active and inactive compound are applied to the leaves of sensitive plants. The leaves may be treated in situ or, alternatively, excised leaves may be used. The relative toxicity of the compounds can be estimated by grading the ensuing damage to the plant tissues and by measuring the size of lesions formed within a given time period. Other known assays can be performed at the cellular level, employing standard tissue culture methodologies e.g., using cell suspension cultures.

By "structurally related mycotoxin" is meant any mycotoxin having a chemical structure related to a fumonisin such as fumonisin B1, for example AAL toxin, fumonisin B2, fumonisin B3, fuimonisin B4, fumonisin C1, fumonisin A1 and A2, and their analogs, as well as other mycotoxins having similar chemical structures that would be expected to be detoxified by activity of the fumonisin degradative enzymes elaborated by *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacteria of ATCC 55552.

By "harvested grain" is meant any form of grain which has been somehow removed from the environment in which it was grown. For example, harvested grain could constitute ear corn, or corn kernels removed from the ear, or cut wheat stalks, or barley or rice kernels, or the like. Harvested grain may be in storage or may be being processed. "Processed grain" is grain that has been through some form of processing and will be used in the production of food for human consumption or will be used as animal feed.

By "transgenic plant" is meant any plant or plant cell that has become transformed by the introduction, stable and heritable incorporation, into the subject plant or plant cell, of foreign DNA, i.e. DNA encoding for a protein not normally found within that plant species. "Plantlet" refers to a plant sufficiently developed to have a shoot and a root that is asexually reproduced by cell culture. "Explant" refers to a section or piece of tissue from any part of a plant for culturing.

By "hormone" is meant any plant growth regulator that affects the growth or differentiation of plant cells. Such hormones include cytokinins, auxins, and gibberellins, as well as other substances capable of affecting plant cells.

The term "callus" and its plural "calli", refer to an unorganized group of cells formed in response to cutting, severing, or other injury inflicted on plant tissue. Excised pieces of plant tissue and isolated cells can be induced to form callus under the appropriate culture conditions. Callus can be maintained in culture for a considerable time by transferring or subculturing parts of the callus to fresh medium at regular intervals. The transfer of callus to liquid medium leads to dispersion of the tissue and the formation of a plant cell suspension culture. Callus can be induced to undergo organized development to form shoots and roots.

"Embryoid" refers to a structure similar in appearance to a plant zygotic embryo.

"Somatic hybrid" and "somatic hybridization" refers generally to stable combination of cellular material, be it protoplast/protoplast or protoplast/cytoplast combinations, and includes cybrids and cybridization.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

As used herein, the term "nucleotide sequence" means a DNA or RNA molecule or sequence, and can include, for example, a cDNA, genomic DNA, or a synthetic DNA sequence, a structural gene or a fragment thereof, or an mRNA sequence that encodes an active or functional polypeptide.

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3'(carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bound at its 3' terminus by the translation start codon (ATG) of a coding sequence and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eucaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Procaryotic promoters contain Shine-Dalgarno sequences.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell.

A coding sequence is "operably linked to" or "under the control of" control sequences in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed., or is capable of undergoing transformation, by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated into (covalently linked to) chromosomal DNA making up the genome of the transformed cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmnid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

Two DNA, RNA or polypeptide sequences are "substantially homologous" when at least about 85 % (preferably at

least about 90 %, and most preferably at least about 95 %) of the nucleotides or amino acids match over a defined length of the molecule. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., supra; DNA Cloning, Vols. I & II, supra; Nucleic Acid Hybridization, supra.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a bacterial gene, the gene will usually be flanked by DNA that does not flank the bacterial gene in the genome of the source bacterium. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). "Heterologous" DNA also refers to DNA not found within the host cell in nature. Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as these terms are used herein.

The term "polypeptide" as used herein is used in its broadest sense, i.e., any polymer of amino acids (dipeptide or greater) linked through peptide bonds. Thus, the term "polypeptide" includes proteins, oligopeptides, protein fragments, analogues, muteins, fusion proteins and the like. The term also encompasses amino acid polymers as described above that include additional non-amino acid moieties. Thus, the term "polypeptide" includes glycoproteins, lipoproteins, phosphoproteins, metalloproteins, nucleoproteins, as well as other conjugated proteins. The term "polypeptide" contemplates polypeptides as defined above that are recombinantly produced, isolated from an appropriate source, or synthesized.

This invention can be better understood by reference to the following non-limiting examples. It will be appreciated by those skilled in the art that other embodiments of the invention may be practiced without departing from the spirit and the scope of the invention as herein disclosed and claimed.

EXAMPLE 1

Chemicals and reagents. All chemicals were reagent grade or better unless otherwise indicated. Fumonisin B_1 and B_2 were obtained from Sigma Chemical Co. Partially purified fumonisins (eluate from C8 column) were obtained from Dr. Pat Murphy (Iowa State University). AAL-toxin (TA isomer) was a gift of Dr. David Gilchrist (University of California-Davis).

Plant tissue samples. Mature, field-grown maize seed was obtained from maize breeding locations of Pioneer Hi-Bred International, Inc. in the Southeast, Midwest and South Central regions of the U.S. Seed was stored at room temperature in individual packets.

Fungal and bacterial isolates. Exophiala and Rhinocladiella isolates from maize were isolated as described below. Other isolates were obtained from Dr. C. J. Wang (Syracuse, N.Y.), Dr. Michael McGinnis (Case Western Reserve University, Cleveland, Ohio), and from the American Type Culture Collection (Bethesda, MD). *Fusarium graminearum* [Gibberella zeae (Schw.) Petsch], *Diplodia maydis*, and *Fusarium moniliforme* Sheld., were obtained from the microbial culture collection of Pioneer Hi-Bred International, Inc. *Aspergillus flavus* (Schw.) Petsch, isolate CP22, was obtained from Don Sumner at the University of Georgia (Tifton, Ga.). Xanthomonas sp. 2412.1 was isolated from maize stalk tissue as described below.

Isolation methods. Individual kernels, either intact or split in two with a sterile razor blade, were rinsed for 1 hr in 5 ml sterile water with agitation. From 1 to 5 µof the rinse fluid was added to 100 µL of sterile, carbon-free mineral salts medium+FB_1 (MS-FB_1) (FB_1) (1 g/liter NH_3 S0$_4$, 1 g/liter K_2HPO_4, 1 g/liter NaCl, 0.2 g/liter $MgSO_47H_2O$, pH 7.0) containing FB_1 (Sigma Chemical Co.) at 0.5 to 1.0 mg/ml). The pH of the medium was approx. 6.0 after addition of FB_1. After 1 to 2 weeks incubation at 28° C. in the dark, serial 10-fold dilutions were made in sterile dH_2O, and aliquots were plated onto 1.2% Bacto-agar containing 0.1% yeast extract, 1% Bacto-peptone and 0.1% dextrose (YPD agar). Fungal and bacterial colonies that appeared on agar were transferred onto fresh plates and individual colonies were evaluated for fumonisin metabolizing ability by inoculating them into fresh MS-FB_1. Loss of fumonisin from the medium was monitored periodically by spotting 0.5 to 1 microliter aliquots of culture supernatant on C_{18} silica gel plates that were then air-dried and developed as described below (see Analysis of fumonisins and metabolism products).

Direct isolation of black yeasts from seed was accomplished by plating 100 microliters of seed wash fluid onto YPD or Sabouraud agar augmented with cycloheximide (500 mg/liter) and chloramphenicol (50 mg/liter). Plates were incubated at room temperature for 7–14 days, and individual pigmented colonies that arose were counted and cultured for analysis of fumonisin-degrading ability as described above.

For stalk isolations, mature stalk samples 0.5×0.5×2 cm were taken from Southern-type maize inbreds grown in Johnston, Iowa by Pioneer Hi-Bred International, Inc., a seed company, in 1993. One-inch sections of the center (pith) or the outside of non-surface-sterilized stalk were cut and placed in 10 ml. sterile water in a small, sterilized tube. The tubes were shaken for 1 hour, and then 2 µ of washate were withdrawn and used to inoculate 100 µof MS-FB_1 in a microtiter plate. Subsequent steps were as above.

Analysis of fumonisins and metabolism products. Analytical thin-layer chromatography was carried out on 100% silanized C_{18} silica plates (Sigma™#T-7020; 10×10 cm; 0.1 mm thick) by a modification of the published method of Rottinghaus. Sample lanes were pre-wet with methanol to facilitate sample application. After application of from 0.1 to 2 µof aqueous sample, the plates were air-dried and developed in MeOH:4% KCl (3:2) or MeOH:0.2M KOH (3:2) and then sprayed successively with 0.1M sodium borate (pH 9.5) and fluorescamine (0.4 mg/ml in acetonitrile). Plates were air-dried and viewed under long wave UV.

Alkaline hydrolysis of FB_1 to AP_1. FB_1 or crude fumonisin C_8 material was suspended in water at 10–100 mg/ml and added to an equal volume of 4N NaOH in a screw-cap tube. The tube was sealed and incubated at 60° C. for 1 hr. The hydrolysate was cooled to RT and mixed with an equal volume of ethyl acetate, centrifuged at 1000 RCF for 5 minute and the organic (upper) layer recovered. The pooled ethyl acetate layers from two successive extractions were dried under N_2 and resuspended in dH_2O. The resulting material (the aminopentol of FB_1 or "AP_1") was analyzed by TLC.

Tables 1 and 2 illustrate the results of efforts to isolate a fumonisin-degrading enzyme from a wide assortment of sources. As is noted, *E. spinifera* isolates from maize seed

from various locations were always able to produce a fumonisin-degrading enzyme when grown on fumonisin as a sole carbon source (Table 1), as were *E. spinifera* isolates from other sources from around the world (Table 2). Some samples of *Rhinocladiella atrovirens* from maize seed were also able to produce this enzyme (Table 1). Other species of Exophiala and other sources and species of Rhinocladiella were routinely unable to produce the enzyme, even when isolated from plant-related sources (Table 2).

TABLE 1

Dematiaceous fungi isolated from maize seed that degrade fumonisin

Isolate#	Species	Location of origin	Isolated from	Appearance[1]	Modification of substrates FB_1	Modification of substrates AP_1
2369.E7	*Exophiala spinifera*	Tifton, GA	Maize seed (3293)	clean	+	+
2369.G5	*Exophiala spinifera*	Tifton, GA	Maize seed (3379)	clean	+	+
2174.A4	*Exophiala spinifera*	Tifton, GA	Maize seed (inbred)	moldy	+	+
2369.F7	*Exophiala spinifera*	Winterville, NC	Maize seed (3170)	moldy	+	+
2369.H9	*Exophiala spinifera*	Winterville, NC	Maize seed (3379)	moldy	+	+
2141.10	*Exophiala spinifera*	Winterville, NC	Maize seed (unk)	moldy	+	+
2174.C6	*Rhinocladiella atrovirens*	Winterville, NC	Maize seed (unk)	moldy	+	+
2170.2	*Exophiala spinifera*	Winterville, NC	Maize seed (inbred)	moldy	+	+
2174.A4	*Exophiala spinifera*	Union City, TN	Maize seed (inbred)	moldy?	+	+
2219.H5	*Exophiala spinifera*	Union City, TN	Maize seed (inbred)	moldy	+	+
2363.1	*Exophiala spinifera*	Weslaco, TX	Maize seed (inbred)	moldy	+	+
2363.3	*Exophiala spinifera*	Weslaco, TX	Maize seed (inbred)	moldy	+	+
2363.3	*Exophiala spinifera*	Weslaco, TX	Maize seed (inbred)	moldy	+	+
2363.8	*Exophiala spinifera*	Weslaco, TX	Maize seed (inbred)	moldy	+	+
2363.10	*Exophiala spinifera*	Weslaco, TX	Maize seed (inbred)	moldy	nt	
2369.F11	*Rhinocladiella atrovirens*	Johnston, IA	Maize seed (inbred)	clean	+	+

[1]"moldy" implies visible discoloration of kernel pericarp, cracking or splitting; "clean" implies no visible signs of infection on the kernel
[2]Evaluated by TLC analysis of culture supernatants as described herein
nt = not tested.

TABLE 2

Other fungal isolates tested for degradation of fumonisin B1 in liquid culture

Isolate	Species	Source	Location of Origin	Isolated from	Modification of substrates FB_1	Modification of substrates AP_1
		Black Yeast Fungi				
26089	*Exophiala spinifera*	ATCC	Uruguay	Palm trunk	+	+
26090	*Exophiala spinifera*	ATCC	Uruguay	Palm tree fruit	+	+
26091	*Exophiala spinifera*	ATCC	Uruguay	Bird's nest	+	+
26092	*Exophiala spinifera*	ATCC	Uruguay	Bird's nest	+	+
48173	*Exophiala spinifera*	ATCC		Nasal Granuloma	+	+
56567	*Exophiala spinifera*	ATCC		?	+	+
18218	*Exophiala spinifera*	ATCC		Nasal Granuloma	+	+
58092	*Exophiala spinifera*	ATCC		Human	+	+
66775	*Exophiala monileae*	ATCC			–	nt
32288	*Exophiala salmonis*	ATCC	Unknown	Leaf Litter	–	nt
26438	*Exophiala pisciphila*	ATCC	Australia	Wheat rhizosphere	–	nt
26272	*Exophiala jeanselmi*	ATCC	Canada	Activated sludge	–	nt
P-154	*Rhinocladiella atrovirens*	C. J. Wang	Chester, NJ	Southern pine pole	–	nt
P-330	*Rhinocladiella atrovirens*	C. J. Wang	Binghamton, NY	Southern pine pole	–	nt
P-646	*Rhinocladiella atrovirens*	C. J. Wang	Virginia	Southern pine pole	–	nt
P-1492	*Rhinocladiella atrovirens*	C. J. Wang	Chester, NJ	Southern pine pole	–	nt
ED-43	*Rhinocladiella atrovirens*	C. J. Wang	Unknown	Douglas-fir pole	–	nt
ED-124	*Rhinocladiella atrovirens*	C. J. Wang	Unknown	Douglas-fir pole	–	nt
28220	*Rhinocladiella anceps*	ATCC	Maryland	Grass	–	nt
		Ear mold fungi				
FMO001	*Fusarium moniliforme*	PHI	Unknown	Maize	–	nt

TABLE 2-continued

Other fungal isolates tested for degradation of fumonisin B1 in liquid culture

Isolate	Species	Source	Location of Origin	Isolated from	Modification of substrates FB_1	Modification of substrates AP_1
FGR001	*Fusarium graminearum*	PHI	Unknown	Maize	–	nt
CP22	*Aspergillus flavus*	PHI	Unknown	Maize	–	nt
DMA001	*Diplodia maydis*	PHI	Unknown	Maize	–	nt

*Tested both with FB_1 and as a sole carbon source and with FB_1 amended with 1% sucrose.

TABLE 3

Frequency of isolation of fumonisin-degrading black yeast isolates from maize seed

Location of origin	# samples tested	# samples positive	% containing FB_1-degrading black yeast	Species identified
Weslaco, TX	8	6	75.0	*Exophiala spinifera*
Winterville, NC	19	4	47.5	*Exophiala spinifera, Rhinocladiella atrovirens*
Tifton, GA	8	3	37.5	*Exophiala spinifera*
Union City, TN	7	2	28.2	*Exophiala spinifera*
Johnston, IA	7	1	14.3	*Rhinocladiella atrovirens*
Shelbyville, IL	3	0	0	none
Macomb, IL	4	0	0	—
Champaign, IL	3	0	0	—
Yale, IN	3	0	0	—
California	8	0	0	—
Total	70	16	22.8	

Organisms can be screened for their ability to degrade fumonisin using the present methods. In this way, plant, soil, marine and fresh water samples can be screened and organisms isolated therefrom that are able to degrade fumonisin. Alternatively, already isolated microbial strains that are suspected of possessing this capability can be screened. Putative fumonisin-resistant bacteria include bacteria associated with plant species susceptible to Fusarium infection. For instance, bacteria associated with Fusarium-infected tomato and pepper as well as other susceptible plant species, might be expected to degrade fumonisin. Furthermore, members of bacterial genera known to be versatile in their catabolism of complex organic molecules, such as members of the genus Pseudomonas, might degrade fumonisin.

Generally, media used to culture the above microbes will contain a known amount of fumonisin, i.e. from 0.1 to 3 mg of fumonisin per ml of media, more usually from 0.25 to 2 mg per ml of media, and preferably from 0.5 to 1 mg of fumonisin per ml of media.

A further study was performed to determine if colony morphology could be used to determine which strains of these species would produce a fumonisin-degrading enzyme. The results as shown in Table 4 indicated that *E spinifera* and *R. atrovirens* colonies having different morphologies could nevertheless produce the fumonisin-degrading enzyme.

TABLE 4

Black yeasts recovered from a single kernel by direct plating seed washates onto YPD + cycloheximide + chloramphenicol[1]

Isolate	Colony Type on YPD agar	Species	# colonies	# FB_1 degr
2403.5	Light brown, shiny	*Exophiala spinifera*	33	33
2403.25	Dark brown, shiny	*Exophiala spinifera*	1	1
2403.12	Brown, velvety	*Rhinocladiella atrovirens*	4	4
2403.2	Grey, velvety	*Rhinocladiella atrovirens*	1	1
Totals			39	39

[1]Kernel source: Tifton, Georgia. Seed was split, washed in 5 ml sterile water and then 100 ul was plated onto YPD agar containing cycloheximide (500 mg/L) and chloramphenicol (50 mg/L).

From these results it was concluded that growth on fumonisin as the sole carbon source is the most reliable indicator of the ability to produce the fumonisin-degrading esterase.

The esterase isolated from *E. spinifera* was then subjected to other treatments, including proteases, to determine whether and how the enzyme would function in various environments. The results are indicated in Table 5.

TABLE 5

Effect of various treatments on modification of FB_1

Treatment	Conditions	FB_1 Hydrolase activity*
Control	16 hr, 37° C., pH 5.2	+++
Boiling water bath	100° C., 30 min, pH 5.2	–
Protease K	0.01 mg/ml, 16 hr, 37° C., pH 5.2	+
Pronase E	0.01 mg/ml, 16 hr, 37° C., pH 5.2	++
Chymotrypsin	0.01 mg/ml, 16 hr, 37° C., pH 5.2	++
Trypsin	0.01 mg/ml, 16 hr, 37° C., pH 5.2	+++
EDTA	50 mM	++
DTT	25 mM	+++
Ca^{++}	50 mM	+++
Mg^{++}	50 mM	+++
PMSF	10 mM	+++

*10-fold concentrated, 11 to 15 day culture filtrates treated as described and then incubated with FB_1 (0.5 mg/ml final conc) overnight at 37° C. Analysis by C_{18} TLC/fluorescamine spray following overnight incubation at 37° C. with 1 mg/ml fumonisin
– = no hydrolysis
± = trace amount of hydrolysis
+ = incomplete hydrolysis
++ = incomplete hydrolysis
+++ = complete hydrolysis

Next, the pH range of activity of the fumonisin esterase was evaluated by measuring fumonisin degradation in the presence of citrate and citrate-phosphate buffers at varying pH levels. Results are shown in Table 6. From this, it was concluded that the pH range of the enzyme was quite wide, and that the enzyme would function at the internal pH of plants and plant cells.

TABLE 6

Effect of buffer pH on hydrolysis of fumonisin B_1 by *E. spinifera* culture filtrate

Buffer	pH	FB_1 Hydrolase activity*
0.1 M citrate	3.0	+++
0.1 M citrate-phosphate	4.0	+++
0.1 M citrate-phosphate	5.0	++
0.1 M citrate-phosphate	6.0	++
0.1 M phosphate	7.0	±
0.1 M phosphate	8.0	–

*reactions were carried out at 37° C. overnight and then assayed by TLC
*Analysis by C_{18} TLC/fluorescamine spray following overnight incubation at 37° C. with 1 mg/ml fumonisin.
– = no hydrolysis
± = trace amount of hydrolysis
+ = incomplete hydrolysis
++ = incomplete hydrolysis
+++ = complete hydrolysis

The fuimonisin esterase isolated from *E. spinifera* and *R. atrovirens* was compared with other known esterases from various sources as supplied by commercial vendors. The results shown in Table 7 indicate that the fimonisin esterase is a unique enzyme that is highly specific in its activity and does not have a generalized esterase activity comparable to that of any of the known enzymes tested.

TABLE 7

Hydrolysis of fumonisin B_1 by commercial esterases and hydrolases

Enzyme	Code	Source, purity	Units/mg prot.	Units per rxn	Assay pH	FB_1 hydrolysis
Esterase, nonspecific	EC 3.1.1.1	Rabbit	100		8.0	–
Esterase, nonspecific	EC 3.1.1.1	Porcine liver	200		7.5	–
Lipase	EC 3.1.1.3	*Candida cylindrica*	35		7.7	–
Cholinesterase, butyryl	EC 3.1.1.8	Horse serum, highly purified	500	15	8.0	–
Cholinesterase, acetyl	EC 3.1.1.7	Bovine, partially pure	0.33	0.15	8.0	–
Cholesterol esterase	EC 3.1.1.13	Bovine, partially pure	0.5	0.15	8.0	–
Cholesterol esterase	EC 3.1.1.13	Porcine, partially pure		0.15	8.0	–
Cholesterol esterase	EC 3.1.1.13	*Pseudomonas fluorescens*	12	1.5	7.0	–
Cholesterol esterase,	EC 3.1.1.13	Pseudomonas sp.	200	15	7.0	±
Acetylesterase	EC 3.1.1.6	Orange Peel partially pure	4	0.15	6.5	–
Pectinesterase	EC 3.1.1.11	Orange Peel, partially pure	100	1.5	7.5	–
Pectinase	EC 3.2.1.15	Rhizopus Crude	0.5	1.5	4.0	–
Pectinase	EC 3.2.1.15	Aspergillus Partially pure	5	0.1	4.0	–

TABLE 7-continued

Hydrolysis of fumonisin B_1 by commercial esterases and hydrolases

Enzyme	Code	Source, purity	Units/mg prot.	Units per rxn	Assay pH	FB_1 hydrolysis
Fumonisin esterase	?	*Exophiala spinifera*, crude	unk	unk	5.2	+++

*Analysis by C_{18} TLC/fluorescamine spray following overnight incubation at 37° C. with 1 mg/ml fumonisin.
– = no hydrolysis
± = trace amount of hydrolysis
+ = incomplete hydrolysis
++ = incomplete hydrolysis
+++ = complete hydrolysis

The enzyme of this invention was evaluated for inducibility by growing an Exophiala culture on various carbon sources of varying degrees of structural similarity to fumonisin. The results, shown in Table 8, illustrate that both the original form of fumonisin and its metabolite are capable of inducing enzyme production, but that inducibility of the enzyme is also quite specific.

TABLE 8

Ability of various carbon sources to support growth and/or induction of FB_1 hydrolytic activity Exophiala culture activity

Carbon source	Concentration	Growth	FB_1 hydrolase activity
FB_1	0.1%	+	+
Alkaline hydrolyzed FB_1 (AP1)	0.1%	+	+
Na+ Tricarballylate	0.1%	±	–
Sphingosine	0.1%	–	–
Phytosphingosine	0.1%	–	–
Na+ Citrate	0.1%	+	–
Sucrose	0.1%	+	–
Glucose	0.1%	+	–

The ability of the fumonisin esterase to cleave other organic carboxylesters was also evaluated in comparison to its ability to hydrolyse fumonisin. The results, shown in Table 8, also illustrate that the enzyme hydrolyzed the tricarballylates of other related aminoalcohols such as FB_2 and AAL toxin.

TABLE 9

Hydrolysis of organic carboxylesters by Exophiala crude concentrated culture filtrate

Substrate	Conditions	Assay method	Hydrolysis by Exophiala culture filtrate
FB_1	pH 5.2, 37° C., 1 hr	C_{18} TLC: fluorescamine	+
FB2	pH 5.2, 37° C., 1 hr	C_{18} TLC: fluorescamine	+
AAL-toxin	pH 5.2, 37° C., 1 hr	C_{18} TLC: fluorescamine	+

Enzyme activity of culture filtrate and myceliurn. *Exophiala spinifera* isolate 2141.10 was grown on YPD agar for 1 week, and conidia were harvested, suspended in sterile water, and used at 105 conidia per ml to inoculate sterile Fries mineral salts medium containing 1 mg/ml purified FB_1 (Sigma Chemical Co.). After 2 weeks incubation at 28° C.

in the dark, cultures were filtered through 0.45 micron cellulose acetate filters, and rinsed with Fries mineral salts. Fungal mycelium was suspended in 15 mL of 0.1 MC-FB_1, pH 5.2+1 mM EDTA+3 μg/ mL Pepstatin A+1.5 μg/mL Leupeptin and disrupted in a Bead Beaterm using™0.5 mm beads and one minute pulses, with ice cooling. Hyphal pieces were collected by filtering through Spin X (0.22 μm), and both mycelial supernatant and original culture filtrates were assayed for fuimonisin modification by methods outlined above.

Preparation of crude culture filtrate. Agar cultures grown as above were used to inoculate YPD broth cultures (500 ml) in conical flasks at a final concentration of 105 cells per ml culture. Cultures were incubated 5 days at 28° C. without agitation and mycelia harvested by filtration through 0.45 micron filters under vacuum. The filtrate was discarded and the mycelial mat was washed and resuspended in sterile carbon-free, low mineral salts medium (1 g/liter NH_3NO_4; 1 g/liter NaH_2P0_4; 0.5 g/liter $MgCl_2$; 0.1 g/liter NaCl; 0.13 g/liter $CaCl_2$; 0.02 g/liter $FeSO_4$ $7H_2O$, pH 4.5) containing 0.5 mg/ml alkaline hydrolyzed crude FB_1. After 3–5 days at 28° C. in the dark with no agitation the cultures were filtered through low protein binding 0.45 micron filters to recover the culture filtrate. Phenylmethyl sulfonyl fluoride (PMSF) was added to a concentration of 2.5 mM and the culture filtrate was concentrated using an Amicon™YM 10 membrane in a stirred cell at room temperature, and resuspended in 50 mM sodium acetate, pH 5.2 containing 10 mM $CaCl_2$. The crude culture filtrate (approx. 200-fold concentrated) was stored at –20° C.

To obtain preparative amounts of enzyme-hydrolyzed fumonisin, 10 mg. of FB_1 (Sigma) was dissolved in 20 mL of 50 mM sodium acetate at pH 5.2+10 mM $CaCl_2$, and 0.25 mL of 200×concentrated crude culture filtrate of 2141.10 was added. The solution was incubated at 37° C. for 14 hours, and then cooled to room temperature. The reaction mixture was brought to approx. pH 9.5 by addition of 0.4 mL of 4N KOH, and the mixture was extracted twice with 10 mL ethyl acetate. The combined organic layers were dried under LN_2 and resuspended in dH_2O. 2.5 milligrams of organic extracted material were analyzed by Fast Atom Bombardment (FAB) mass spectrometry. The resulting mass spectrum showed a major ion at M/2=406 mass units, indicating the major product of enzymatic hydrolysis was AP_1, which has a calculated molecular weight of 406.63.

Additional characterization of fumonisin esterases from Exophiala spinifera and Gram-negative bacterium species. Crude, concentrated culture filtrates (induced for FB_1 esterase activity) from *E. spinifera* isolate 2141.10 and Xanthomonas sp. 2412.1 were chromatographed on a Pharmaciag® Superdex 75 size exclusion column and eluted with 50 mM sodium phosphate, pH 6.0 containing 0.2M NaCl. One-mL fractions were collected and assayed for FBesterase activity by methods described above. The retention times for the 2141.10 and 2412.1 FB_1 esterases resulted in estimated molecular weights of 44.5 and 28.7 kilodaltons, respectively.

Similarly, crude concentrated culture filtrates in 1.7M ammonium sulfate were injected onto a Pharmacia® Phenyl Sepharose FPLC column equilibrated with 1.7M ammonium sulfate in 50 mM sodium phosphate pH 6.0 (Buffer A). A 30 mL, linear gradient of Buffer A to distilled water was applied, followed by a wash with 0.1% Triton X-100 in. 50 mM sodium phosphate, pH 6.0. One-mL factions were collected and assayed for both FB1 esterase and for non-specific esterase (as measured by napthyl acetate hydrolysis using the method of Dary et al. (1990) "Microplate adaptation of Gomori's assay for quantitative determination." Journal of Economic Entomology 83: 2187–2192. FIG. 2*a* and *b* shows the retention times for the specific (i.e. FB_1) versus nonspecific (i.e. naphthyl acetate esterase) activities. Both fungal and bacterial FB_1 esterase activity eluted at approx. 0.4M ammonium sulfate. Naphthyl acetate esterase activity was detected in both fungal and bacterial cultures but this activity did not co-elute with the FB_1 esterase activity. Thus the fungal and bacterial FB_1 esterases are not the same as nonspecific esterases detectable in the culture filtrates of these microbes.

EXAMPLE 2

Cloning of genes coding for fumonfisin esterase

Microorganisms demonstrating fumonisin-resistance can be used to create a genomic library using standard techniques, well known in the art. Thus, restriction enzymes can be used to render DNA fragments which can in turn be inserted into any number of suitable cloning vectors. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. The cloning vector need only be capable of transforming a host cell incapable of fumonisin degradation. Examples of recombinant DNA vectors for cloning and host cells which they can transform, shown in parentheses, include the bacteriophage lambda (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFRI (gram-negative bacteria), pvE290 (non-*E. coli* gram-negative bacteria), pIJ61 (Streptomyces), pUC6 (Streptomyces), YIp5 (Saccharomyces), and YCp19 (Saccharomyces). See, generally DNA Cloning, Vols. I and II, supra, and Maniatis et al., supra. Particularly useful is a cloning vector able to transform *E. coli*.

Once the cloning vector has been inserted into an appropriate host cell, the cells are grown on fumonisin containing media and screened for their ability to degrade fumonisin as previously described. Plasmid DNA inserts from colonies that degrade fumonisin are characterized by subdloning, transposon tagging, and DNA sequence analysis, all well within the skill in the art (see, e.g., Napoli, C., and Staskawicz, B. (1987) J. Bact. 169:572–578). Once a coding sequence is determined, recombinant protein molecules able to degrade fumonisin can be produced according to the present invention by constructing an expression cassette and transforming a. host cell therewith to provide a cell line or culture capable of expressing the desired protein which is encoded within the expression cassette.

Sequences encoding the fuimonisin degradation enzyme can be either prepared directly by synthetic methods based on the determined sequence, or by using the sequence to design oligonucleotide probes to clone the native coding sequence using known techniques. The oligonucleotide probes can be prepared and used to screen a DNA library from an organism able to degrade fumonisin as determined above. The basic strategies for preparing oligonucleotide probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See, e.g., DNA Cloning, Vol. I, supra; Nucleic Acid Hybridization, supra; Oligonucleotide Synthesis, supra; Maniatis et al., supra.

The coding sequence can be comprised entirely of the coding sequence so derived, or such sequences can be fused to other sequences (e.g., leader sequences) so that a fusion protein is encoded. See, e.g., U.S. Pat. Nos. 4,431,739 ;

4,425,437 and 4,338,397, the disclosures of which are hereby incorporated by reference. Once an appropriate coding sequence for the fumonisin-degrading enzyme has been prepared or isolated, it can be cloned into any suitable vector or replicon, known in the art. These vectors are described above, with *E. coli* being the host bacterium particularly preferred.

To complete construction of the expression cassettes, the coding sequence is then operably linked to control sequences such as a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator, so that the DNA sequence encoding the protein is transcribed into messenger RNA in the host cell transformed by the vector containing the expression construction. It is within the skill of the art to operably link the fumonisin-degrading enzyme coding sequence to appropriate control sequences in order to bring about transcription and translation. In general, the coding sequence will be downstream from the promoter sequence and any expression regulatory regions, such as enhancers or operator sequences. If the coding sequence is linked to a heterologous coding sequence or start codon, then it is important to place the coding sequence in reading frame with the latter. If the intended expression host is procaryotic, then it will also be necessary to include a ribosome binding site among the upstream control sequences. Downstream operably linked control sequences will usually comprise a transcription termination sequence.

The construct can then be inserted into an appropriate expression vector. A number of procaryotic and eucaryotic expression vectors are known in the art. Preferred vectors are procaryotic expression vectors. A particularly preferred host for such vectors is *E. coli*. The fumonisin-degrading enzyme is then produced by growing the host cells transformed by the expression cassette under conditions which cause the expression of the biologically active protein, as indicated by the host cells ability to degrade fumonisin in the medium on which it is grown, as described above. The protein can be isolated from the host cells and purified for further study. If the protein is not secreted, it may be necessary to disrupt the host cells and purify the protein from the cellular lysate. Various purification techniques, such as HPLC, size-exclusion chromatography, electrophoresis, and immunoaffinity chromatography, are known, and the selection of the appropriate purification and recovery method is within the skill of the art.

Similarly, the gene can be inserted into the T-DNA region of a Ti or Ri plasmid derived from *A. tumefaciens* or *A. rhizogenes*, respectively. Thus, expression cassettes can be constructed as above, using these plasmids. Many control sequences are known which when coupled to a heterologous coding sequence and transformed into a host organism show fidelity in gene expression with respect to tissue/organ specificity of the original coding sequence. See, e.g., Benfey, P. N., and Chua, N. H. (1989) Science 244: 174–181. Particularly suitable control sequences for use in these plasmids are promoters for constitutive leaf-specific expression of the gene in the various target plants. Other useful control sequences include a promoter and terminator from the nopaline synthase gene (NOS). The NOS promoter and terminator are present in the plasmid pARC2, available from the American Type Culture Collection and designated ATCC 67238. If such a system is used, the virulence (vir) gene from either the Ti or Ri plasmid must also be present, either along with the T-DNA portion, or via a binary system where the vir gene is present on a separate vector. Such systems, vectors for use therein, and methods of transforming plant cells are described in U.S. Pat. No. 4,658,082; U.S. application Ser. No. 913,914, filed Oct. 1, 1986, as referenced in U.S. Pat. 5,262,306, issued Nov. 16, 1993 to Robeson, et al.; and Simpson, R. B., et al. (1986) Plant Mol. Biol. 6: 403–415 (also referenced in the '306 patent); all incorporated by reference in their entirety.

Once constructed, these plasmids can be placed into *A. rhizogenes* or *A. tumefaciens* and these vectors used to transform cells of plant species which are ordinarily susceptible to Fusarium or Alternaira infection. For example, non-resistant varieties of tomato (Lycopersicon esculentum) are often plagued with such infection and new resistant varieties could be developed to withstand Alternaria-induced disease in emerging tomato seedlings, although it should be noted that fusarium wilt in tomato is thought to be caused by *F. oxysoporum*, not *F. monicliforium*, and *F. oxysoporum* apparently does not produce fumonisin. Several other transgenic plants are also contemplated by the present invention including but not limited to soybean, corn, sorghum, alfalfa, rice, clover, cabbage, banana, coffee, celery, tobacco, cowpea, cotton, melon and pepper. The selection of either A. *tumefaciens* or *A. rhizogenes* will depend on the plant being transformed thereby. In general *A. tumefaciens* is the preferred organism for transformation. Most dicotyledons, some gymnosperms, and a few monocotyledons (e.g. certain members of the Liliales and Arales) are susceptible to infection with *A. tumefaciens*. *A. rhizogenes* also has a wide host range, embracing most dicots and some gymnosperms, which includes members of the Leguminosae, Compositae and Chenopodiaceae. Alternative techniques which have proven to be effective in genetically transforming plants include particle bombardment and electroporation. See e.g. Rhodes, C. A., et al. (1988) Science 240, 204–207; Shigekawa, K. and Dower, W. J. (1988) BioTechniques 6, 742–751; Sanford, J. C., et al. (1987) Particulate Science & Technology 5:27–37; and McCabe, D. E. (1988) BioTechnology 6:923–926.

Once transformed, these cells can be used to regenerate transgenic plants, capable of degrading fumonisin. For example, whole plants can be infected with these vectors by wounding the plant and then introducing the vector into the wound site. Any part of the plant can be wounded, including leaves, stems and roots Alternatively, plant tissue, in the form of an explant, such as cotyledonary tissue or leaf disks, can be inoculated with these vectors and cultured under conditions which promote plant regeneration. Roots or shoots transformed by inoculation of plant tissue with *A. rhizogenes* or *A. tumefaciens*, containing the gene coding for the fumonisin degradation enzyme, can be used as a source of plant tissue to regenerate fumonisin-resistant transgenic plants, either via somatic embryogenesis or organogenesis. Examples of such methods for regenerating plant tissue are disclosed in Shahin, E. A. (1985) Theor. Appl. Genet. 69:235–240; U.S. Pat. No. 4,658,082; Simpson, R. B., et al. (1986) Plant Mol. Biol. 6: 403–415; and U.S. Patent applications Ser. Nos. 913,913 and 913,914, both filed Oct. 1, 1986, as referenced in U.S. Pat. No. 5,262,306, issued Nov. 16, 1993 to Robeson, et al.; the entire disclosures therein incorporated herein by reference.

Such transformed cells can also be used to regenerate transgenic plants capable of expressing, in specific tissues or constitiuatively, depending upon the type of promoter utilized, either the fumonisin degrading enzymes elaborated by *Exophiala spinefera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the 55552, or the AP1, catabolase elaborated by those strains. Such transgenic plants can be harvested, and the appropriate tissues (seed, for example, if a seed specific promoter were used) can be subjected to

large scale protein extraction and purification techniques, and the fumonisin degradation enzymes or AP_1 catabolases can be isolated for use in fumonisin and fumonisin hydrolysis product detoxification processes.

Certain esterases fall into a family that is related by primary sequence and overall structure (Cygler M., Schrag J. D., Sussman J. L., Harel M., Silman I., Gentry M. K., Doctor B. P. (1993) "Relationship between sequence conservation and 3-Dimensional structure in a large family of esterases, lipases, and related proteins." Protein Sci 2: 366–382.). PCR primers were designed based on highly conserved regions of this esterase family and using these primers, a cDNA clone from *Exophiala spinifera* isolate 2141.10 was obtained that showed significant homology to known esterases, and wis specifically induced by fumonisin and other inducers. This esterase can be expressed in *E. coli* and its enzyme activity can be measured by means of the TLC assay described above. If no activity is obtained in *E. coli* then expression can be measured in yeast or another eukaryotic system.

Other methods can also be used to clone the gene. Purification of the protein and N-terminal sequencing allow design of specific DNA probes; generation of antibodies from purified protein and screening an expression library; using RNA enrichment methods to obtain cDNAs specific to the induced culture. Once the gene has been confirmed as corresponding to fumonisin esterase, the cDNA clone can easily be ligated into appropriate expression vectors for expression of the enzyme in maize tissue culture cells, transgenic maize, and also in *Fusarium moniliforme* itself, that is useful for studying the mechanisms of pathogenesis associated with the fungus and its toxin. Transformed or transient-expressing maize tissue culture cells can then be evaluated for resistance to fumonisins relative to control transformed tissue, and in fact fumonisin can be used as a selection agent to isolate transformed cells from tissue culture.

Cloning of XanthomonasI/Sphingomonas Esterase Gene

The Xanthomonas esterase gene was cloned in a lambda ZAP express expression library from Sau3 A partially digested bacterial DNA (4–8 kb size selected from ATCC 55552). Pools of lambda lysates were tested for fumonisin esterase assay by TLC using pure fumonisin as a substrate, and positive pools were sub-sampled to enrich for positive clones. Individual plaques were resuspended and activity assayed in the lysate. One positive clone was purified, phagemid excised and DNA prepared for sequencing. A 4 kilobase DNA fragment containing fumonisin esterase activity was sequenced and found to contain a 1589 base pair region containing a 529 amino acid open reading frame with high homology to members of the serine carboxylesterase type B superfamily. The open reading framecodes for a hypothetical protein (called BEST1) with a putative signal peptide from amino acid 1 to 38, giving a mature protein with a calculated molecular weight of 51,495.63 daltons and a pI of 8.19. This open reading frame showed 52.5 % similarity and 34% identity with the amino acid sequence of a rabbit cholinesterase (P37176). Other cholinesterases showed similar homology scores. The BEST1 sequence was also 53.0% similar and 36.4% identical to a Bacillus subtilis para-nitrobenzyl esterase (P04058). The open reading frame also showed 54.6% similarity and 34.9% identity with the *Exophiala spinifera* fumonisin esterase (Esp 1). Aside from their overall similarity with other type B carboxylesterases, Esp1 and BEST1 share two short amino acid domains not found in other known esterases of this type:

Protein	Sequence	From	To
ESP1	ATLM	292	295
BEST1	ATLM	286	289
ESP1	TNI	175	177
BEST1	TNI	172	174

These domains may be involved in the substrate specificity of these enzymes (Cygler M., Schrag, J.D., Sussman, J. L. Harel, M., Silman I., Gentry, M. K. Doctor, B. P. (1993) Relationship between sequence conservation and 3-Dimensional structure in a large family of esterases, lipases, and related proteins. Protein Sci. 2:366–382.).

EXAMPLE 3

Preparation of AP1-induced and non-induced mycelium

Exophiala spinifera isolate 2141.10 was grown in YPD broth for 5 days at 28° C., mycelium was harvested on 05. micron cellulose acetate filters and transferred to fresh medium consisting of Fries mineral salts (Gilchrist D. G., Grogan R. G. (1976) "Production and nature of a host-specific toxin from *Alternaria alternata* f.sp. *lycopersici*." Phytopathology 66: 165–171) amended with hydrolyzed fumonisin B1 (AP1) (0.5 mg/mL) or delta-aminobutyric acid (δ-ABA) (1 mg/mL) as the sole carbon source. Cultures were incubated in the dark for 48 hr at 28° C. and culture supernatants removed by filtration through 0.5 micron cellulose acetate. The remaining mycelial mat was washed with sterile Fries mineral salts and then frozen in liquid nitrogen for storage.

EXAMPLE 4

RNA isolation from *Exophiala spintfera*

The mycelial mats described above (~1 gram) were ground in liquid nitrogen in a mortar and pestle following addition of 10 mL "TRIREAGENT"(Molecular Research Center, Inc. Cincinnati, Ohio) in the presence of 0.2 volume chloroform. The grindate was centrifuged and the resulting supernatant precipitated with isopropanol. The resulting pellet was extracted with phenol, ethanol precipitated, and stored at –80° C.

The RNA in water (0.4 mL) was enriched for poly-A-containing mRNA using biotin-oligo(dT) and a streptavidin magnetic bead system (Promega) using the manufacture's instructions. The polyA(+)-enriched RNA was stored at–800°C.

First strand cDNA synthesis from polyA(+)-enriched RNA was carried out using M-MLV reverse transcriptase (37° C., 1 hr). The reaction mixture was extracted with phenol and chloroform. Aliquots were taken for polymerase chain reaction (PCR) using the degenerate primers identified in SEQUENCE I.D. NOS. 1 through 4. :

ESP5'-OL1
GGGGAATTCGARGAYTGNYTNTAYNTNAAYRT
(SEQUENCE I.D. NO. 1)

ESP5'-OL2
GGGGAATTCMCNGTNNTNVTNTGGATNYAYGGNGGNG
(SEQUENCE I.D. NO. 2)

-continued

ESP3'-OL1
GGGAAGCTTGGRTYNCCNCCRAANKBNGCDATRTT
(SEQUENCE I.D. NO. 3)

ESP3'-OL2
GGGAAGCTTCNCCNGCNSWYTCNCCRAANADNGTNA
(SEQUENCE I.D. NO. 4)

Most bases designated "N" were inosines.

Thermocycler reaction conditions were:

1. 94° C. 2min
2. 94° C. 30 sec
3. 45° C. 2 min
4. 72° C. 1 min
5. repeat steps 2–4 for 35×
6. 72° C. 5 min

The PCR reaction products were electrophoresed on horizontal agarose gels. Bands that were present only in induced lanes were excised and the DNA was eluted. The recovered DNA was digested with HindIII and EcoRI and ligated into pBluescript SK+. A recombinant clone from products amplified using ESP5'-OL2 and ESP3'-OL2 (ESP26-1) was recovered and sequenced. The cloned region contains an open reading frame with the partial protein or amino acid sequence . . .

S F H L Y D G A S F A A N Q D V I V V T I N Y R T-
N I L G F P A A P Q L P I T Q R N L G F L D Q R F A L D W V
Q R N I A A F G G D P R K V T FFGESA. . . (SEQUENCE I.D. NO. 5)

The above deduced amino acid sequence from DNA fragment ESP26- 1 showed significant homology to a family of proteins that includes cholinesterases, acetylcholinesterases, carboxylesterases, and certain lipases (Cygler M., Schrag J. D., Sussman J. L., Harel M., Silman L, Gentry M. K., Doctor B. P. (1993) "Relationship between sequence conservation and 3-Dimensional structure in a large family of esterases, lipases, and related proteins." Protein Sci 2: 366–382.)

EXAMPLES 5–6

Comparison of Deduced Amino Acid Sequence to Known Sequences

In comparison with a sequence published in Arpagaus, M., Chatonnet, A., Masson, P., Newton, M., Vaughan, T. A., Bartels, C. F., Nogueira, C. P., La Du, B. N., and Lockridge, O. J. Biol. Chem. 266, 6966–6974 (1991), 43 of the 76 amino acids in ESP26-1 were identical to a dog pancreatic cholinesterase.

In another comparison 32 of 62 amino acids from ESP26-1 were identical to a fungal lipase, as published by Lotti, M., Grandori, R., Fusetti, F., Longhi, S., Brocca, S., Tramontano, A., and Alberghina, L., Gene 124, 45–55 (1993).

EXAMPLE 7

Northern blot analysis of induced, non-induced *Exophiala spinifera*

Total RNA extracted from *Exophiala spinifera* cultures as described in the preceding examples was electrophoresed on agarose gels containing formaldeyde, blotted to nitrocellulose, and probed with random-primed 32P-loabelled ESP26-1 cDNA. The probe hybridized to an RNA of approximately 2.0 kilobases in size in the induced lane, but not in the non-induced lane (see FIG. 1).

EXAMPLE 8

Isolation of full length cDNA of ESP26-1 from *Exophiala spinifera.*

To obtain 3'-end of the cDNA coding for the putative esterase, a 3'rapid amplification of cDNA ends protocol (3'-RACE) was employed (Frohman, M. A., Dush, M. K., and Martin, G. R. 1988 "Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer." Proc. Natl. Acad. Sci. 85: 8998–9002). 5μg of total RNA isolated from AP1 induced *Exophiala spinifera.* mycelia was used as template for reverse transcription reaction. The reverse transcription reaction and subsequent PCR amplification was performed with a 3'-RACE kit (Gibco BRL). The gene-specific primer (ESP3'-1: GCTAGTTTCGCAGCCAATCAGGA) (SEQUENCE I.D. NO. 6) was designed based on ESP26-1 sequence. PCR reaction conditions were:

1. 94° C. 4 min
2. 94° C. 45 sec
3. 60° C. 25 sec
4. 72° C. 3 min
5. repeat steps 2–4 for 40×
6. 72° C. 10 min

A resulting 1.5 kb DNA fragment was blotted to nitrocellulose and hybridized with cDNA ESP26-1 under highly stringent hybridization and wash conditions (last wash: 0.1 X SSC, 0.5% SDS, 65° C. for 30 min.) The DNA fragment was gel-isolated, ligated into a pGEM-T vector (Promega), and transformed into DH5α (Gibco BRL). The resulting plasmid DNA (p3RC-2) was sequenced using M13 universal primer. Sequence comparison of 3RC-2 and ESP26-1 indicated the ESP26-1 overlapped 100% with the 5' end of 3RC-2 sequence.

To obtain the amino-terminal sequence, a 5'-RACE strategy was employed (Frohman, et al, supra). 5 μg of total RNA isolated from AP1 induced *Exophiala spinifera.mycelia* was reverse transcribed with SuperScript I RNase H-reverse Transcriptase (Gibco BRL) using an anti-sense primer constructed against ESP26-1 sequence (ESP5'-1: AAAGGCTGCGATGTTCCGCTGTA) (SEQUENCE I.D. NO. 7). The cDNA was tailed with dATP using terminal transferase (Promega) and used as a template for nested amplification using a second gene-specific anti-sense primer (ESP5'-2: TCGCTGTGTTATTGGCAGCTGAG. (SEQUENCE I.D. NO. 8). C was a silent mutation of A in order to create a Pvu II restriction site) and an end-blocked polyT primer (BamT17V: CGCGGATCCGTTTTTTTTTTTTTTTV) (SEQUENCE I.D. NO. 9).

PCR reaction conditions were:

1. 94° C. 4 min
2. 94° C. 45 sec
3. 40° C. 45 sec
4. 60° C. 25 sec
5. 72° C. 3 min
6. repeat steps 2–5 for 41×
7. 72° C. 10 min

The PCR products were fractionated on a 1.5 % agarose gel. The amplified product was gel-isolated, ligated into

pGEM-T (Promega), and transformed into DH5 (Gibco BRL). The resulting 5' RACE product was sequenced and shown to overlap as expected with the 3' RACE product and to contain an open reading frame with significant homology to members of the serine esterase/lipase superfamily described by Cygler et al. (supra). The overlapping sequences obtained by 3' RACE and 5' RACE were combined to yield a cDNA sequence corresponding to the complete open reading frame. The full length, 1937 bp cDNA clone from *Exophiala spinifera* 2 141.10 (abbreviated ESP1) contains an open reading frame of 537 amino acids as shown below (SEQUENCE I.D. NO. 10).

MPSRYILSWLLTCFLGIAFGSRCGSSAPTVKIDAGMVVGTTTTVPGTTATVSEFLG
VPFAASPTRFAPPTRPVPWSTPLQATAYGPACPQQFNYPEELRETTMAWFNTPPPSA
GESEDCLNLNIYVPGTENTNKAVMVWIYGGALEYGWNSFHLYDGASFAANQDVI
VVTINYRTNILGFPAAPQLPITQRNLGFLDQRFALDWVQRNIAAFGGDPRKVTIFG
QSAGGRSVDVLLTSMPHNPPFRAAIMESGVANYNFPKGDLSEPWNTTVQALNCT
TSIDILSCMRRVDLATLMNTIEQLGLGFEYTLDNVTVVYRSETARTTGDIARVPVL
VGTVANDGLLFVLGENDTQAYLEEAIPNQPDLYQTLLGAYPIGSPGIGSPQDQIAAI
ETEVRFQCPSAIVAQDSRNRGIPSWRYYYNATFENLELFPGSEVYHSSEVGMVFGT
YPVASATALEAQTSKYMQGAWAAFAKNPMNGPGWKQVPNVAALGSPGKAIQVD
VSPATIDQRCALYTHYYTELGTIAPRTF

This open reading frame (ORF) shows some homology to members of the serine esterase/lipase superfamily described by Cygler et al. (supra). The most extensive homology is 35.9% identity in 320 amino acid overlap with butyrylcholinesterase from *Oryctolagus cuniculus* (rabbit).

The deduced Esp1 protein contains a putative signal peptide which is probably cleaved at position 26/27 yielding a. mature protein with a calculated MW of 54953.781 and calculated pI of 4.5. These calculated values are consistent with the estimated MR and pI of the fumonisin esterase activity described above.

A comparison of the Esp 1 open reading frame consensus regions in the esterase superfamily (Cygler et al., supra) reveals numerous conserved features indicating Esp1 may code for a serine esterase. The Esp protein has a potential serine active site consensus at 223–228; a putative aspartate active site consensus at 335–341 that is typical of cholesterol esterases and Drosophila 6 and P proteins [the majority of members of this superfamily, including fungal lipases and carboxylesterases have glutamate at the active site instead of aspartate]; and a putative histidine active site that is different from any members of the family, containing additional amino acids between the G and H. The putative Esp mature protein has a total of 6 cysteines, for 3 possible disulfide bridges, consistent with at least a subset of the esterases in the superfamily described by Cygler et al., supra

Thus the Esp ORF has most of the hallmarks of a bona fide member of the lipase/esterase superfamily, including a putative active site triad and other conserved amino acids. The regions of conservation are not consistent with any one substrate subgroup (i.e. lipase, cholinesterase, carboxylesterase, or cholesterol esterase), but seem to be contain some features of several of these, and Esp appears to be unique among known esterases in its putative active site His consensus sequence.

EXAMPLE 9

Effect of FB_1 and AP1 on maize coleoptiles

Maize coleoptiles from 4 day dark-grown germinated maize seeds were excised above the growing point and placed in 96-well microtiter plates in the presence of 60 microliters of sterile distilled water containing FB_1 or AP_1 at approximately equimolar concentrations of 1.5, 0.5, 0.15, 0.05, 0.015, 0.005, 0.0015, or 0.0005 millimolar, along with water controls. After 2 days in the dark at 28° C. the coleoptiles were placed in the light and incubated another 3 days. Injury or lack thereof was evaluated as follows:

	0	.0005	.0015	.005	.015	.05	.15	.5	1.5	mM
FB_1	–	–	–	–	+/–	+	+	+	+	
AP1	–	–	–	–	–	–	–	–	+	

\+ = brown necrotic discoloration of coleoptile
– = no symptoms (same as water control)

The results (see table above) indicate there is at least a 30-fold difference in toxicity between FB_1 and AP_1 to maize coleoptiles of this genotype. This is in general agreement with other studies where the toxicity of the two compounds was compared for plant tissues: In Lemna tissues, AP_1 was approx. 40-fold less toxic (Vesonder R. F., Peterson R. E., Labeda D., Abbas H. K. (1992) "Comparative phytotoxicity of the fumonisins, AAL-Toxin and yeast sphingolipids in Lemna minor L (Duckweed)." Arch Environ Contam Toxicol 23: 464–467.). Studies with both AAL toxin and FB_1 in tomato also indicate the hydrolyzed version of the molecule is much less toxic (Gilchrist D. G., Ward B., Moussato V., Mirocha C. J. (1992) "Genetic and Physiological Response to Fumonisin and AAL-Toxin by Intact Tissue of a Higher Plant." Mycopathologia 117: 57–64.). In a recent report Lamprecht et al. also observed an approximate 100-fold reduction in toxicity to tomato by AP_1 versus FB_1 (Lamprecht S., Marasas W., Alberts J., Cawood M., Gelderblom W. Shephard G., Thiel P., Calitz J. (1994) Phytotoxicity of fumonisins and TA-toxin to corn and tomato. Phytopathology 84: 383391.)

EXAMPLE 10

Effect of FB_1 and AP_1 on maize tissue cultured cells (Black Mexican Sweet, BMS)

FB_1 or AP_1 at various concentrations was added to suspensions of BMS cells growing in liquid culture medium in 96-well polystyrene plates. After 1 week the cell density in wells was observed under low power magnification and growth of toxin-treated wells was compared to control wells that received water. Growth of BMS cells was significantly inhibited at 0.4 micromolar FB_1 but no inhibition was observed until 40 micromolar AP_1. This represents an approximate 100-fold difference in toxicity to maize tissue cultured cells. Similarly Van Asch et al. (Vanasch A. J.,

Rijkenberg F. H. J., Coutinho T. A. (1992) "Phytotoxicity of fumonisin b1, moniliformin, and t-2 toxin to corn callus cultures." Phytopathology 82: 1330–1332) observed significant inhibition of maize callus grown on solid medium at 1.4 micromolar. AP_1, was not tested in that study, however.

EXAMPLE 11

AP_1Catabolase Activity

A cell-free extract that contains the catabolase activity was obtained by subjecting substrate-induced *Exophiala spinifera* cells to disruption using a bead beater in sodium acetate buffer, pH 5.2, and recovering the cell-free supernatant by centrifugation and 0.45 micron filtration. Catabolic activity is assayed by incubating extracts with AP_1 (hydrolyzed fumonisin B_1, backbone) or 14C-labelled with the extract and evaluating by TLC on C18 silica. The product AP_1-N1 has a lower Rf than $AP_1$and is detected either by radiolabel scan or by H_2SO_4 spray/charring of the TLC plate. AP_1-N_1does not react with the amine reagent, fluorescamine, that is routinely used to detect AP_1on TLC plates, suggesting that the amine group is missing or chemically modified. Activity is greater at 37° C. than at room temperature, but following 30 min. at 65° C. or 100° C. (no AP_1 catabolic activity remained). Activity is maximal at pH to 9. At pH 9, complete conversion to Ap_1-N_1 occurred in 30 minutes. Activity is retained by 30,000 dalton molecular weight cutoff membrane, but only partially retained by 100,000 dalton molecular weight cutoff membrane. Other amine-containing substrates were tested for modification by the crude extract fumonisin (with tricarboxylic acids attached) is not modified by the extract, indicating that hydrolysis must occur first for the catabolase to be active. Other long-chain bases (sphingosine, sphinganine, phytosphingosine) are apparently not modified by the crude catabolase, suggesting the enzyme(s) is specific for the fumonisin backbone. Preparative amounts of the product, tentatively named AP_1-N_1have also been purified and analyzed by C13 nmr. The results indicate that AP_1-N_1lack an amino nitrogen, and that it probably contains a keto function. This may point to either an amine oxidase or an ammonia lyase enzyme. The product of either enzyme would not be expected to display any significant toxicity (although this has not been tested).

EXAMPLE 12

Demonstration of Functional Esterase Activity

Demonstration of esterase activity was accomplished by expressing the *E. spinifera* cDNA in two heterologous systems (an insect cell/baculovirus expression system, and transgenic maize obtained by microprojectile bombardment) and subsequently detecting fumonisn esterase activity in transformed tissue. Forty-four maize calli (Hi type II) were bombarded with the esterase gene fused to the ubiquitin promoter plus a selectable marker (PAT) plasmid. Using thin layer chromatographic analysis, 38 lines were positive for fumonisin hydrolysis based upon the presence of a free tricarboxylic acid spot in the TLC plates. Four negative control calli had no such spot. Similar results were obtained with insect cells infected with a baculovirus expression vector containing the esterase clone.

Esterase activity was also detected in leaf tissue of regenerated TO maize plants. Ten leaf punches (4 mm dia) were taken from the 6th leaf of regenerated plants of roughly the same age (7 leaf stage) representing four classes of transformants: selectable marker (Bar) only, low esterase (+), medium esterase (++) and high esterase (+++) expressors (based on callus data). Four plants, representing different events in each class, were sampled. The punches were suspended in 200 microliters of 50 mM sodium acetate buffer, pH 5.2, containing leupeptin and pepstatin to inhibit proteinases, and homogenized by rapid agitation with a 5/32" steel bead, twice for 30 sec. The homogenates were spun at 4000 rpm, 10 min. at 4C and 150 microliters supernatant recovered. Samples were assayed for protein concentration (Bradford assay) and adjusted to the same protein concentration and then assayed for fumonisin esterase activity using 14C-FB1 as a substrate. After 30 min. or 4 hrs. reactions were spotted on TLC plates (C18) and developed with MeOH:4%KC1 (3:2). Plates were scanned with a radiometric scanner (AMBIS) and scored for fumonisin esterase activity on a product), ++ (up to 50% conversion to product) and +++ (between 90–100% conversion to product).

SAMPLE	CALLUS SCORE (30 MIN)	30 MIN. LEAF SCORE	4 HR. LEAF SCORE
A1	+	–	–
B1 Control	–	–	–
C1	++	–	–
D1	+	–	+
E1 Control	–	–	–
F1	+++	+	++
G1	+++	+	+++
H1	++	+	++
A2	+	–	–
B2 Control	–	–	–
C2 Control	–	–	–
D2	+++	+++	+++
E2	++	+	++
F2	–	–	–
G2	+++	+	+++
H2	+++	+	+++

In summary, 8 or 12 callus expressors were positive for leaf expression. All negative controls plus four callus expressors were negative. Of the four "+++" callus expressors, only one (D2) had the same high level (30 min. assay), but all were positive.

EXAMPLE 13

Detoxification of Harveste(I Grain

The present invention also relates to a method of detoxifying a fumonisin or a structurally related mycotoxin with an enzyme having the structure of the fumonisin degradative enzymes or the AP_1 catabolase elaborated by *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atravirens*, ATCC 74210, or the bacterium of ATCC 55552 during the processing of grain for animal or human food consumption. Since the atmospheric ammoniation of corn has proven to be an ineffective method of detoxification (see B. Fitch Haumann, "Eradicating Mycotoxin in Food and Feeds," INFORM 6:248–257(1995)), such a methodology is particularly critical where transgenic detoxification is not applicable.

In this embodiment, the fumonisin degradative enzyme and/or the AP_1 catabolase elaborated by *Expohiliala spinfera*, ATCC 74269, Rhinocladiella atravirens, ATCC 74210, or the bacterium of ATCC 55552, are presented to grain during the processing procedure, at the appropriate stages of the procedure and in amounts effective for detoxification of fumonisins and structurally related mycotoxins. Detoxification by this method can occur not only during the

grain processing, but also any time prior to feeding of the grain to an animal or incorporation of the grain into a human food product.

The enzymes can be introduced during processing in appropriate manners, for example as a wash or spray, or in dried or lyophilized form or powered form, depending upon the nature of the milling process and/or the stage of processing at which the enzymatic treatment is carried out. See generally, Hoseney, R. C., *Principles of Cereal Science and Technology*, American Assn. of Cereal Chemists, Inc., 1990 (especially Chapters 5, 6 and 7); Jones, J. M., Food Safety, Eagan Press, St. Paul, Minn., 1992 (especially Chapters 7 and 9); and Jelen, P., *Introduction to Food Processing*, Restan Publ. Co., Reston, Va., 1985. Processed grain to beeffective amount of the enzy treated with an effective amount of the enzymes in the form of an inoculant or probiotic additive, for example, or in any form recognized by those skilled in the art for use in animal feed. The enzymes of the present invention are expected to be particularly useful in detoxification during processing and/or in animal feed prior to its use, since the enzymes display relatively broad ranges of pH activity. The esterase from *Exophilia spinfera*, ATCC 74269, showed a range of activity from about pH 3 to about pH 6, and the esterase from the bacterium of ATCC 55552 showed a range of activity from about pH 6 to about pH 9.

EXAMPLE 14

Genetic Engineering of Ruminal Microorganisms

Ruminal microorganisms can be genetically engineered to contain and express either the fumonisin degrading enzymes or the AP1 catabolase elaborated by *Exophilia spinfera*, ATCC 74269, *Rhinocladiella atravirens*, ATCC 74270, or the bacterium of ATCC 55552, or a combination of the enzymes. The genetic engineering of microorganisms is now an art recognized technique, and ruminal microorganisms so engineered can be added to feed in any art recognized manner, for example as a probiotic or inoculant. In addition, microorganisms capable of functioning as bioreactors can be engineered so as to be capable of mass producing either the fumonisin degrading enzymes or the AP_1, catabolase elaborated by *Exophilia spinfera*, ATCC 74269, *Rhinocladiella atravirens*, ATCC 74270, or the bacterium of ATCC 55552.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 12

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGGAATTCG ARGAYTGNYT NTAYNTNAAY RT 32

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGGGAATTCM CNGTNNTNVT NTGGATNYAY GGNGGNG 37

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGAAGCTTG GRTYNCCNCC RAANKBNGCD ATRTT 35

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs

-continued

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGGAAGCTTC NCCNGCNSWY TCNCCRAANA DNGTNA 36

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Phe His Leu Tyr Asp Gly Ala Ser Phe Ala Ala Asn Gln Asp Val
1 5 10 15

Ile Val Val Thr Ile Asn Tyr Arg Thr Asn Ile Leu Gly Phe Pro Ala
20 25 30

Ala Pro Gln Leu Pro Ile Thr Gln Arg Asn Leu Gly Phe Leu Asp Gln
35 40 45

Arg Phe Ala Leu Asp Trp Val Gln Arg Asn Ile Ala Ala Phe Gly Gly
50 55 60

Asp Pro Arg Lys Val Thr Phe Phe Gly Glu Ser Ala
65 70 75

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCTAGTTTCG CAGCCAATCA GGA 23

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAAGGCTGCG ATGTTCCGCT GTA 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCGCTGTGTT ATTGGCAGCT GAG 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGCGGATCCG TTTTTTTTTT TTTTTTTV 28

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 527 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Pro Ser Arg Tyr Ile Leu Ser Trp Leu Leu Thr Cys Phe Leu Gly
1 5 10 15

Ile Ala Phe Gly Ser Arg Cys Gly Ser Ser Ala Pro Thr Val Lys Ile
20 25 30

Asp Ala Gly Met Val Val Gly Thr Thr Thr Thr Val Pro Gly Thr Thr
35 40 45

Ala Thr Val Ser Glu Phe Leu Gly Val Pro Phe Ala Ala Ser Pro Thr
50 55 60

Arg Phe Ala Pro Pro Thr Arg Pro Val Pro Trp Ser Thr Pro Leu Gln
65 70 75 80

Ala Thr Ala Tyr Gly Pro Ala Cys Pro Gln Gln Phe Asn Tyr Pro Glu
85 90 95

Glu Leu Arg Glu Ile Thr Met Ala Trp Phe Asn Thr Pro Pro Pro Ser
100 105 110

Ala Gly Glu Ser Glu Asp Cys Leu Asn Leu Asn Ile Tyr Val Pro Gly
115 120 125

Thr Glu Asn Thr Asn Lys Ala Val Met Val Trp Ile Tyr Gly Gly Ala
130 135 140

Leu Glu Tyr Gly Trp Asn Ser Phe His Leu Tyr Asp Gly Ala Ser Phe
145 150 155 160

Ala Ala Asn Gln Asp Val Ile Val Val Thr Ile Asn Tyr Arg Thr Asn
165 170 175

Ile Leu Gly Phe Pro Ala Ala Pro Gln Leu Pro Ile Thr Gln Arg Asn
180 185 190

Leu Gly Phe Leu Asp Gln Arg Phe Ala Leu Asp Trp Val Gln Arg Asn
195 200 205

Ile Ala Ala Phe Gly Gly Asp Pro Arg Lys Val Thr Ile Phe Gly Gln
210 215 220

Ser Ala Gly Gly Arg Ser Val Asp Val Leu Leu Thr Ser Met Pro His
225 230 235 240

Asn Pro Pro Phe Arg Ala Ala Ile Met Glu Ser Gly Val Ala Asn Tyr
245 250 255

Asn Phe Pro Lys Gly Asp Leu Ser Glu Pro Trp Asn Thr Thr Val Gln
260 265 270

Ala Leu Asn Cys Thr Thr Ser Ile Asp Ile Leu Ser Cys Met Arg Arg
275 280 285

Val Asp Leu Ala Thr Leu Met Asn Thr Ile Glu Gln Leu Gly Leu Gly
290 295 300

Phe Glu Tyr Thr Leu Asp Asn Val Thr Val Val Tyr Arg Ser Glu Thr
305 310 315 320

Ala Arg Thr Thr Gly Asp Ile Ala Arg Val Pro Val Leu Val Gly Thr
325 330 335

-continued

Val	Ala	Asn	Asp	Gly	Leu	Leu	Phe	Val	Leu	Gly	Glu	Asn	Asp	Thr	Gln
			340					345					350		
Ala	Tyr	Leu	Glu	Glu	Ala	Ile	Pro	Asn	Gln	Pro	Asp	Leu	Tyr	Gln	Thr
		355					360					365			
Leu	Leu	Gly	Ala	Tyr	Pro	Ile	Gly	Ser	Pro	Gly	Ile	Gly	Ser	Pro	Gln
	370					375					380				
Asp	Gln	Ile	Ala	Ala	Ile	Glu	Thr	Glu	Val	Arg	Phe	Gln	Cys	Pro	Ser
385					390					395					400
Ala	Ile	Val	Ala	Gln	Asp	Ser	Arg	Asn	Arg	Gly	Ile	Pro	Ser	Trp	Arg
				405					410					415	
Tyr	Tyr	Tyr	Asn	Ala	Thr	Phe	Glu	Asn	Leu	Glu	Leu	Phe	Pro	Gly	Ser
			420					425					430		
Glu	Val	Tyr	His	Ser	Ser	Glu	Val	Gly	Met	Val	Phe	Gly	Thr	Tyr	Pro
		435					440					445			
Val	Ala	Ser	Ala	Thr	Ala	Leu	Glu	Ala	Gln	Thr	Ser	Lys	Tyr	Met	Gln
	450					455					460				
Gly	Ala	Trp	Ala	Ala	Phe	Ala	Lys	Asn	Pro	Met	Asn	Gly	Pro	Gly	Trp
465					470					475					480
Lys	Gln	Val	Pro	Asn	Val	Ala	Ala	Leu	Gly	Ser	Pro	Gly	Lys	Ala	Ile
				485					490					495	
Gln	Val	Asp	Val	Ser	Pro	Ala	Thr	Ile	Asp	Gln	Arg	Cys	Ala	Leu	Tyr
			500					505					510		
Thr	His	Tyr	Tyr	Thr	Glu	Leu	Gly	Thr	Ile	Ala	Pro	Arg	Thr	Phe	
		515					520					525			

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1800 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACTAGTGGAT CATTGCATTG GCTGGCGGAC TGGCGCGCCG ATAGTCGTTG CGATGGTCGC 60

GAGAATAAGC GTGCGAAGTG GGAGGATGTG AAGATGGGGG CCAGGAGTAT GTGTGCGGGA 120

CGGTTCGGAC GCTTCTGCAT TGGCTTGGCT TCATCGGTTG CCGTGACTCT AGGGGGAGCC 180

TCCGCCGCCG GCGCGGCAAC CGCGACGGAT TTTCCGGTCC GCAGGACCGA TCTGGGCCAG 240

GTTCAGGGAC TGGCCGGGGA CGTGATGAGC TTTCGCGGAA TACCCTATGC AGCGCCGCCG 300

GTGGGCGGGC TGCGTTGGAA GCCGCCCCAA CACGCCCGGC CCTGGGCGGG CGTTCGCCCC 360

GCCACCCAAT TTGGCTCCGA CTGCTTCGGC GCGGCCTATC TTCGCAAAGG CAGCCTCGCC 420

CCCGGCGTGA GCGAGGACTG TCTTTACCTC AACGTATGGG CGCCGTCAGG CGCTAAACCC 480

GGCCAGTACC CCGTCATGGT CTGGGTCTAC GGCGGCGGCT TCGCCGGCGG CACGGCCGCC 540

ATGCCCTACT ACGACGGCGA GGCGCTTGCG CGACAGGGCG TCGTCGTGGT GACGTTTAAC 600

TATCGGACGA ACATCCTGGG CTTTTTCGCC CATCCTGGTC TCTCGCGCGA GAGCCCCACC 660

GGAACTTCGG GCAACTACGG CCTACTCGAC ATTCTCGCCG CTCTTCGGTG GGTGCAGAGC 720

AACGCCCGCG CCTTCGGAGG GGACCCCGGC CGAGTGACGG TCTTTGGTGA ATCGGCCGGA 780

GCGAGCGCGA TCGGACTTCT GCTCACCTCG CCGCTGAGCA AGGGTCTCTT CCGTGGCGCT 840

ATCCTCGAAA GTCCAGGGCT GACGCGACCG CTCGCGACGC TCGCCGACAG CGCCGCCTCG 900

GGCGAGCGCC TCGACGCCGA TCTTTCGCGA CTGCGCTCGA CCGACCCAGC CACCCTGATG 960

GCGCGCGCCG ACGCGGCCCG CCCGGCATCG CGGGACCTGC GCAGGCCGCG TCCGACCGGA 1020

-continued

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CCGATCGTCG ATGGCCATGT GCTGCCGCAG ACCGACAGCG CGGCGATCGC GGCGGGGCAG   1080
CTGGCGCCGG TTCGGGTCCT GATCGGAACC AATGCCGACG AAGGCCGCGC CTTCCTCGGG   1140
CGCGCGCCGA TGGAGACGCC AGCGGACTAC CAAGCCTATC TGGAGGCGCA GTTTGGCGAC   1200
CAAGCCGCCG CCGTGGCGGC GTGCTATCCC CTCGACGGCC GGGCCACGCC CAAGGAAATG   1260
GTCGCGCGCA TCTTCGGCGA CAATCAGTTC AATCGGGGGG TCTCGGCCTT CTCGGAAGCG   1320
CTTGTGCGCC AGGGCGCGCC CGTGTGGCGT TATCAGTTCA ACGGTAATAC CGAGGGTGGA   1380
AGAGCGCCGG CTACCCACGG AGCCGAAATT CCCTACGTTT TCGGGGTGTT CAAGCTCGAC   1440
GAGTTGGGTC TGTTCGATTG GCCGCCCGAG GGGCCCACGC CCGCCGACCG TGCGCTGGGC   1500
CAACTGATGT CCTCCGCCTG GGTCCGGTTC GCCAAGAATG GCGACCCCGC CGGGGACGCC   1560
CTTACCTGGC CTGCCTATTC TACGGGCAAG TCGACCATGA CATTCGGTCC CGAGGGCCGC   1620
GCGGCGGTGG TGTCGCCCGG ACCTTCCATC CCCCCTTGCG CGGATGGCGC CAAGGCGGGG   1680
TGACGCCGTC GACGATGGCG TGACGACGGT CGAGGCGATG TTCTCGATCT GGAGTCCGCG   1740
CCGCCTCGAT TTGCGTCGTC TCCGGCGCTC AGACGAACGC CCCAGTTCCA TCCACACAGT   1800
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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 529 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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Met Gly Ala Arg Ser Met Cys Ala Gly Arg Phe Gly Arg Phe Cys Ile
1               5                  10                  15

Gly Leu Ala Ser Ser Val Ala Val Thr Leu Gly Gly Ala Ser Ala Ala
            20                  25                  30

Gly Ala Ala Thr Ala Thr Asp Phe Pro Val Arg Arg Thr Asp Leu Gly
        35                  40                  45

Gln Val Gln Gly Leu Ala Gly Asp Val Met Ser Phe Arg Gly Ile Pro
    50                  55                  60

Tyr Ala Ala Pro Pro Val Gly Gly Leu Arg Trp Lys Pro Pro Gln His
65                  70                  75                  80

Ala Arg Pro Trp Ala Gly Val Arg Pro Ala Thr Gln Phe Gly Ser Asp
                85                  90                  95

Cys Phe Gly Ala Ala Tyr Leu Arg Lys Gly Ser Leu Ala Pro Gly Val
            100                 105                 110

Ser Glu Asp Cys Leu Tyr Leu Asn Val Trp Ala Pro Ser Gly Ala Lys
        115                 120                 125

Pro Gly Gln Tyr Pro Val Met Val Trp Val Tyr Gly Gly Gly Phe Ala
    130                 135                 140

Gly Gly Thr Ala Ala Met Pro Tyr Tyr Asp Gly Glu Ala Leu Ala Arg
145                 150                 155                 160

Gln Gly Val Val Val Val Thr Phe Asn Tyr Arg Thr Asn Ile Leu Gly
                165                 170                 175

Phe Phe Ala His Pro Gly Leu Ser Arg Glu Ser Pro Thr Gly Thr Ser
            180                 185                 190

Gly Asn Tyr Gly Leu Leu Asp Ile Leu Ala Ala Leu Arg Trp Val Gln
        195                 200                 205

Ser Asn Ala Arg Ala Phe Gly Gly Asp Pro Gly Arg Val Thr Val Phe
    210                 215                 220
```

-continued

Gly	Glu	Ser	Ala	Gly	Ala	Ser	Ala	Ile	Gly	Leu	Leu	Leu	Thr	Ser	Pro	
225					230					235					240	
Leu	Ser	Lys	Gly	Leu	Phe	Arg	Gly	Ala	Ile	Leu	Glu	Ser	Pro	Gly	Leu	
				245					250					255		
Thr	Arg	Pro	Leu	Ala	Thr	Leu	Ala	Asp	Ser	Ala	Ala	Ser	Gly	Glu	Arg	
			260					265					270			
Leu	Asp	Ala	Asp	Leu	Ser	Arg	Leu	Arg	Ser	Thr	Asp	Pro	Ala	Thr	Leu	
		275					280					285				
Met	Ala	Arg	Ala	Asp	Ala	Ala	Arg	Pro	Ala	Ser	Arg	Asp	Leu	Arg	Arg	
	290					295					300					
Pro	Arg	Pro	Thr	Gly	Pro	Ile	Val	Asp	Gly	His	Val	Leu	Pro	Gln	Thr	
305					310					315					320	
Asp	Ser	Ala	Ala	Ile	Ala	Ala	Gly	Gln	Leu	Ala	Pro	Val	Arg	Val	Leu	
				325					330					335		
Ile	Gly	Thr	Asn	Ala	Asp	Glu	Gly	Arg	Ala	Phe	Leu	Gly	Arg	Ala	Pro	
			340					345					350			
Met	Glu	Thr	Pro	Ala	Asp	Tyr	Gln	Ala	Tyr	Leu	Glu	Ala	Gln	Phe	Gly	
		355					360					365				
Asp	Gln	Ala	Ala	Ala	Val	Ala	Ala	Cys	Tyr	Pro	Leu	Asp	Gly	Arg	Ala	
	370					375					380					
Thr	Pro	Lys	Glu	Met	Val	Ala	Arg	Ile	Phe	Gly	Asp	Asn	Gln	Phe	Asn	
385					390					395					400	
Arg	Gly	Val	Ser	Ala	Phe	Ser	Glu	Ala	Leu	Val	Arg	Gln	Gly	Ala	Pro	
				405					410					415		
Val	Trp	Arg	Tyr	Gln	Phe	Asn	Gly	Asn	Thr	Glu	Gly	Gly	Arg	Ala	Pro	
			420					425					430			
Ala	Thr	His	Gly	Ala	Glu	Ile	Pro	Tyr	Val	Phe	Gly	Val	Phe	Lys	Leu	
		435					440					445				
Asp	Glu	Leu	Gly	Leu	Phe	Asp	Trp	Pro	Pro	Glu	Gly	Pro	Thr	Pro	Ala	
	450					455					460					
Asp	Arg	Ala	Leu	Gly	Gln	Leu	Met	Ser	Ser	Ala	Trp	Val	Arg	Phe	Ala	
465					470					475					480	
Lys	Asn	Gly	Asp	Pro	Ala	Gly	Asp	Ala	Leu	Thr	Trp	Pro	Ala	Tyr	Ser	
				485					490					495		
Thr	Gly	Lys	Ser	Thr	Met	Thr	Phe	Gly	Pro	Glu	Gly	Arg	Ala	Ala	Val	
			500					505					510			
Val	Ser	Pro	Gly	Pro	Ser	Ile	Pro	Pro	Cys	Ala	Asp	Gly	Ala	Lys	Ala	
		515					520					525				
Gly																

What is claimed is:

1. A transgenic plant capable of expressing the fumonisin degradative enzyme elaborated by *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium of ATCC 55552.

2. A transgenic plant capable of expressing the AP_1 catabolase elaborated by *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium of ATCC 55552.

3. A method of producing the fumonisin degradative enzyme elaborated by *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium of ATCC 55552, the method comprising producing a transgenic plant which expresses one or more of said fumonisin degradative enzymes and isolating and purifying the enzymes from the plant tissues expressing the enzymes.

4. A method of producing the AP_1 catabolase elaborated by *Exophiala spinifera*, ATCC 74269, *Rhinocladiella atrovirens*, ATCC 74270, or the bacterium of ATCC 55552, the method comprising producing a transgenic plant which expresses more of said AP_1 catabolases and isolating and purifying the enzymes from the plant tissues expressing the enzymes.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO : 5,792,931

DATED : Aug. 11, 1998

INVENTOR(S) : Jonathan Duvick; Tracy Rood; Joyce R. Maddox, and Xun Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 40, Line 59

expresses one or more of said AP_1 catabolases and isolating and

Signed and Sealed this

First Day of December, 1998

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks